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Official Organ of
The American Phytopathological Society

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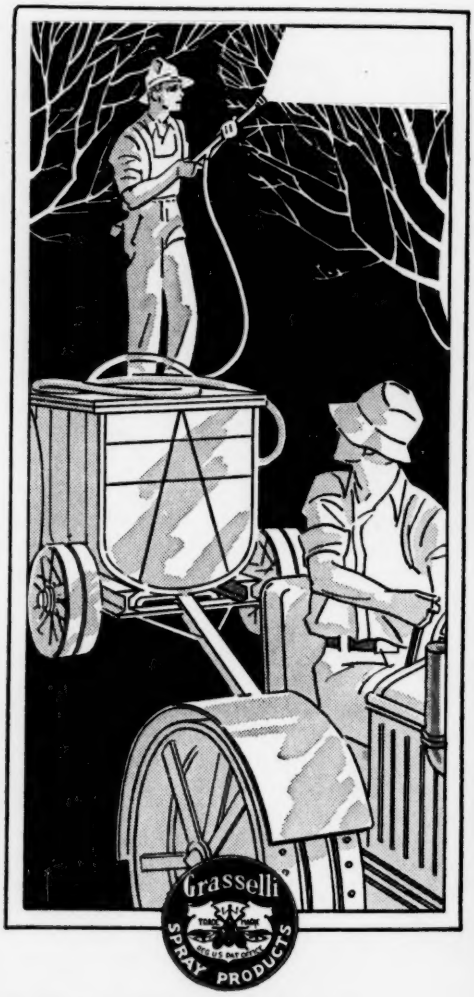

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PHYTOPATHOLOGY

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PHYTOPATHOLOGY

VOLUME 22 FEBRUARY, 1932 NUMBER 2

PHYSIOLOGIC SPECIALIZATION AS A FACTOR IN THE EPIPHYTOLOGY OF PUCCINIA GRAMINIS TRITICI[1]

JAMES M. WALLACE[2]

INTRODUCTION

The phenomenon of physiologic specialization within *Puccinia graminis* Pers. was definitely established by Eriksson (2) in 1894. Stakman and Piemeisel (17), in 1916, demonstrated that further specialization existed within *P. graminis tritici* Eriks. and Henn. Since that time, physiologic specialization has been clearly demonstrated both in *P. graminis avenae* Eriks. and Henn. (14) and *P. graminis secalis* Eriks. and Henn. (6).

The varieties of *Puccinia graminis* now known to be present on the North American Continent, with the degree of physiologic specialization within each variety, so far demonstrated, are as follows:

1. *P. graminis tritici*	Over 100 physiologic forms identified, about 60 of which have been found in nature in North America (13, 9, 15, and unpublished results by Stakman and Levine).

[1] Cooperative investigations of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, and the Minnesota Agricultural Experiment Station.

Presented as a thesis to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy; granted June 15, 1929.

[2] Formerly, Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture. The writer acknowledges with gratitude the helpful suggestions and kindness of Dr. E. C. Stakman, Professor of Plant Pathology, University of Minnesota, and Pathologist, Bureau of Plant Industry, United States Department of Agriculture, under whom this study was conducted. To Dr. M. N. Levine, Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, the writer is indebted for the helpful criticism and invaluable assistance rendered throughout the study. Thanks are due to Mr. Lee Hines, Graduate Assistant, University of Minnesota, for assistance in the inoculation and care of the rust cultures. Finally, to Dr. J. G. Leach, Associate Professor, Department of Plant Pathology, University of Minnesota; Dr. Helen Hart, Assistant Plant Pathologist, Minnesota Agricultural Experiment Station, and Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture; and Mr. Clyde Allison, Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, the writer is under obligation for reading and editing this manuscript.

2. *P. graminis avenae*	7 physiologic forms identified; 5 have been found in North American (14, 1, 3, and 18).
3. *P. graminis secalis*	14 physiologic forms identified, all except one from North American collections (6 and unpublished results by Cotter and Levine).
4. *P. graminis phleipratensis* (Eriks. and Henn.) Stak. and Piem.	Some indication of the existence of at least 2 distinct forms, according to unpublished results of Cotter.
5. *P. graminis agrostidis* Eriks.	Physiologic specialization not demonstrated.
6. *P. graminis poae* Eriks and Henn.	No definitely established evidence of physiologic forms.

In the study of the epiphytology of stem rust in the United States a question of major importance is that of the spring source of inoculum. Studies over a long period of years continue to support the conclusion that urediniospores of *Puccinia graminis tritici* do not, in any important sense, survive the winters in the hard-red-spring-wheat area. What, then, is responsible for the occasional, rather general, and sometimes severe, epidemics of stem rust in this region? Is the primary inoculum still being produced in sufficient quantity on the remaining barberries to cause a general epidemic or is the migration of rust from southern areas the critical factor? It seems plausible that in the years when severe epiphytotics occur one or both of these sources may be important.

This paper reports primarily a study to determine the possible migration of stem rust from the South to the North. The study is based on the annual physiologic-form surveys of 1926, 1927, and 1928, supervised by the writer, who was stationed at University Farm, St. Paul, Minn., during these years. Surveys of this kind have been made each year since 1918 by Stakman and Levine.

Annual physiologic-form surveys show to a certain extent which forms occur in the South and which in the North. An indication is obtained of the prevalence and distribution of forms in these respective regions. The annual survey, then, should be one of the most satisfactory criteria in determining whether stem rust migrates from South to North.

It is obvious that knowledge of the existence of physiologic forms of wheat stem rust and of the prevalence and distribution of such forms is essential to the development of resistant wheat varieties.

The fact that new forms of *Puccinia graminis tritici* are rather frequently making their appearance or, at least, are being discovered empha-

sizes the importance of the surveys in the breeding of resistant wheat varieties. Again, if certain physiologic forms of *P. graminis tritici* are outstandingly predominant in a given region year after year, those forms are the ones to be primarily considered in a breeding program. It is important also to know if other forms occasionally occur in this particular region, even though they be comparatively uncommon. Further, it is of value to know as much as possible of the distribution of all existing forms. In spite of the fact that certain forms, isolated only from widely separated regions, seem relatively scarce, there is no reason to assume that these forms may not at some time in the future become generally distributed.

The surveys of 1926, 1927, and 1928 were similar to those made prior to 1926 by Stakman and Levine,[3] and add certain information of interest and value to wheat breeders. The last three surveys, however, were made with the idea of studying particularly the possibility of the migration of rust from southern fields to those in the North. A careful analysis has been made of the data obtained, and this paper reports the conclusions reached regarding the source of inoculum in each of the 3 years and presents a discussion of the evidence for or against migration of stem rust of wheat from the South to the North.

EXPERIMENTAL MATERIALS AND METHODS

The methods used in making the surveys of 1926, 1927, and 1928 were similar to those used in the surveys made by Bailey (1), Levine (5), Newton, Johnson, and Brown (8), and Stakman *et al.* (13, 16). Collections of stem rust were obtained from the field and from uniform rust nurseries.[4] Uredinial material was collected as early as possible in the spring and collections were continued until the disappearance of that stage in the fall. Collections from the uniform rust nurseries were made by the person who visited the nurseries to take notes on the rust infection of the wheat and oat varieties. The field material was collected by state leaders and scouts of the barberry eradication forces and by other rust workers. The rust was cultured and form identifications made in the plant pathology greenhouses at University Farm, St. Paul, Minnesota.

Differential hosts. The identification of physiologic forms of *Puccinia graminis tritici* is based on the reaction of 12 varieties of wheat that were selected by Stakman and Levine (13) and designated as "differential hosts." These 12 varieties are listed in table 1.

Inoculation and cultural methods. Upon receipt of uredinial material, inoculations were made as promptly as possible. When sufficient rust was available, the 12 differential varieties were inoculated from the original col-

[3] Stakman and Levine—unpublished data.

[4] Nurseries maintained cooperatively by the U. S. Department of Agriculture and various agricultural experiment stations over the United States and eastern Canada to test the reaction of wheat and oat varieties to stem and leaf rusts (5).

lection. However, in most instances it was necessary first to increase the inoculum by inoculating Little Club wheat, a variety susceptible to every form so far discovered in North America. The inoculation and incubation methods were essentially the same as those described by Stakman and Piemeisel (17). The seedlings of the differential varieties were grown in a secluded section of the greenhouse. When about 7 or 8 days old they were inoculated by applying a small quantity of urediniospores to the lower surface of each seedling leaf. A small flat needle was used for transferring the rust from the original material or increase plants to the differential varieties. An alcohol lamp was used for sterilization of the needle. The plants to be inoculated were sprayed with an atomizer filled with tap water so as to induce the spores to stick to the leaves. Usually, the plants had not developed any secondary leaves when inoculated but, if secondary leaves were present, they were clipped off and only the primary leaves inoculated. Immediately after inoculation the plants were incubated for approximately 48 hours in large moist chambers. Upon removal from the chambers, the pots were placed in individual booths on the greenhouse benches. From 15 to 25 plants of each variety were inoculated. All plants not inoculated and those resulting from seed germinating after incubation were removed from the pot. The best possible precautions were taken at all times to prevent accidental infection or contamination.

Identification of physiologic forms. Readings could be made usually after 10 or 12 days, depending somewhat on the amount of sunlight during the developmental period of the rust. The types and degrees of infection described by Stakman and Levine (13) were used.

After all differential varieties were inoculated and had shown clearly their reaction to a particular collection of stem rust, the identity of the latter was determined by the use of the key shown in table 2. This key for the identification of physiologic forms of *Puccinia graminis tritici* is a revision of that of Stakman and Levine (13) and furnished by them. Many physiologic forms can be identified with this key without considering the reaction of the differential varieties. However, the reaction of all the varieties is always determined before deciding upon the identity of a particular physiologic form. For that reason Stakman and Levine prepared a table that gives the variations and constants in the reaction of each differential variety to each physiologic form of *P. graminis tritici.* Since this table was prepared in 1922, it has been extended so as to include the reactions of all forms discovered subsequent to that time. Several foreign forms not known to occur in North America also are included in this key. These data are shown in table 3.

The identification of the physiologic form or forms is not always so simple as one might be led to think from reading a description of the routine

followed in making an identification. Often there may be 2, 3, or sometimes even more physiologic forms in the same field collection. This necessitates the isolation of each form and the determination of its identity separately from the others. In some cases the forms in a mixture can be identified quite easily. For instance, if there are 2 physiologic forms present that react differently on Marquis, 1 form producing a type-2 infection and the other a type-4, and if there is no evidence of differences on any other variety, no additional inoculations are necessary. The 2 forms may be identified immediately, since it is clear that they are identical in all respects except for the differences on Marquis. The key then will show what these 2 forms are.

A rather simple mixture, yet one which would require some additional inoculations, might consist of 2 forms that differ on Marquis and also in their actions on several durum varieties, 1 form perhaps producing a type-4 infection on these differentials and the other only distinct flecks. It would be clear that there were 2 forms, 1 that attacked Marquis and 1 to which Marquis was resistant; likewise, 1 to which the durum varieties were susceptible and 1 to which they were resistant. But it would not be known whether the form to which Marquis was resistant was producing the weak type of infection or the strong type on the durums. Cross inoculations are then necessary in order to make the correct identifications. There may be far more complex mixtures than those cited above; cultures often have been carried for a year or more before final determinations could be made.

TABLE 1.—*List of differential varieties, with their Cereal Investigations numbers and abbreviations, used in identifying physiologic forms of Puccinia graminis tritici*

Triticum compactum	
Little Club, C. I. 4066	Lc
Triticum vulgare	
Marquis, C. I. 3641	Ma
Kanred, C. I. 5146	Krd
Kota, C. I. 5878	Ko
Triticum durum	
Arnautka, C. I. 1493	Arn
Mindum, C. I. 5296	Mnd
Spelmar, C. I. 6236	SpM
Kubanka, C. I. 2094	Kub
Acme, C. I. 5284	Ac
Triticum monococcum	
Einkorn, C. I. 2433	Enk
Triticum dicoccum	
Vernal, C. I. 3686	Ver
Khapli, C. I. 4013	Kpl

TABLE 2.—*Analytical key for the identification of physiologic forms of Puccinia graminis tritici on the basis of their parasitic behavior on differential varieties within the genus Triticum*

Infection homogeneous	
Little Club resistant	Form 41
Little Club susceptible	
Marquis resistant	
Kanred resistant	
Kota resistant	
Arnautka resistant	
Kubanka resistant	Form 2
Kubanka susceptible	
Einkorn resistant	Form 27
Einkorn susceptible	Form 23
Arnautka susceptible	
Mindum resistant	Form 6
Mindum susceptible	
Kubanka resistant	Form 4
Kubanka susceptible	
Einkorn resistant	Form 16
Einkorn susceptible	
Vernal resistant	Form 14
Vernal susceptible	Form 53
Kota susceptible	
Mindum resistant	Form 28
Mindum susceptible	Form 19
Kanred susceptible	
Kota resistant	
Arnautka resistant	
Kubanka resistant	Form 7
Kubanka susceptible	
Vernal resistant	Form 33
Vernal susceptible	Form 51
Arnautka susceptible	Form 10
Kota susceptible	Form 39
Marquis susceptible	
Kanred resistant	
Kota resistant	
Arnautka resistant	
Kubanka resistant	Form 54
Kubanka susceptible	Form 44
Arnautka susceptible	
Acme resistant	Form 46
Acme susceptible	
Khapli resistant	Form 24
Khapli susceptible	Form 42
Kota susceptible	
Arnautka resistant	
Vernal resistant	Form 1
Vernal susceptible	Form 57
Arnautka susceptible	
Mindum resistant	Form 26
Mindum susceptible	
Kubanka resistant	
Vernal resistant	Form 5
Vernal susceptible	Form 8
Kubanka susceptible	
Einkorn resistant	Form 21

TABLE 2—(*Continued*)

Einkorn susceptible	
Vernal resistant	Form 17
Vernal susceptible	Form 9
Kanred susceptible	
Kota resistant	Form 35
Kota susceptible	
Arnautka resistant	
Mindum resistant	
Kubanka resistant	Form 3
Kubanka susceptible	
Acme resistant	Form 20
Acme susceptible	
Einkorn resistant	Form 56
Einkorn susceptible	Form 18
Mindum susceptible	
Spelmar resistant	Form 25
Spelmar susceptible	Form 22
Arnautka susceptible	
Mindum resistant	Form 12
Mindum susceptible	
Kubanka resistant	Form 13
Kubanka susceptible	
Einkorn resistant	
Vernal resistant	Form 34
Vernal susceptible	Form 40
Einkorn susceptible	
Vernal resistant	Form 11
Vernal susceptible	Form 15
Infection heterogeneous	
Marquis resistant	
Kanred resistant	
Arnautka resistant	Form 50
Arnautka susceptible	Form 45
Arnautka indeterminate	
Acme susceptible	Form 48
Acme indeterminate	Form 47
Kanred susceptible	Form 38
Marquis susceptible	
Kanred resistant	
Kota resistant	
Arnautka resistant	Form 43
Arnautka susceptible	Form 55
Kota susceptible	
Arnautka resistant	Form 49
Arnautka susceptible	Form 37
Arnautka indeterminate	
Vernal resistant	Form 29
Vernal susceptible	Form 30
Kanred susceptible	
Kota resistant	Form 31
Kota susceptible	
Arnautka resistant	
Einkorn susceptible	
Vernal resistant	Form 36
Vernal susceptible	Form 52
Arnautka indeterminate	Form 32

TABLE 3.—*Mean infections produced by physiologic forms of Puccinia graminis tritici on differential varieties of Triticum spp.*

Physiologic forms	Reaction of differential varieties[a]											
	Little Club	Marquis	Kanred	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
1	4	4–	0	3+	1=	1	1=	3+	3++	3	0;	1=
2	4	2=	2=	2=	1–	1	1=	1+	3++	3+	1–	0;
3	4	4–	4=	3+	1=	1=	1–	1+	3++	3+	1=	0;
4	4+	2–	1–	2=	4=	3+	3+	2	3++	3++	1=	1=
5	4	4–	0;	3	4=	3++	3++	1++	3+	3	0;	0.
6	4	2	1=	0;	3+	2=	2=	1	3+	3	0.	0;
7	4	2=	3+	1=	1=	1++	1–	1	3++	3–	1	1–
8	4	4	0.	4–	4=	3++	4=	0;	3	3	4	0;
9	4	4–	0	3++	4–	4=	4=	4=	3++	3+	4=	1–
10	4+	2–	3++	2	4	4	4	3++	4–	3+	1=	1=
11	4–	4=	3++	3+	4=	4=	4=	3++	3++	3	1=	1=
12	4+	4–	4=	3+	4=	1	1++	1++	3++	3+	1=	0;
13	4	4–	3++	3++	4=	3++	3++	2–	3++	3	1	1=
14	4+	2–	1–	1++	3++	3++	3++	3++	3++	3	1=	0;
15	4	4–	4=	3++	4=	4=	4=	3++	3++	3+	4=	1=
16	4–	2=	0	1	3++	3+	3++	3–	4=	1=	1=	1
17	4	4–	0.	3+	4=	4=	4=	3++	3++	3	1=	1=
18	4	4–	4=	3++	1	1=	1–	3++	3++	3	1–	1=
19	4	2–	0;	3–	4=	4=	4=	3++	3++	3	0;	1=
20	4	4=	4=	4=	1++	1–	1++	3++	1++	3+	1=	1–
21	4	4	0	3++	4–	4–	4–	4=	3++	1=	0;	1=
22	4+	4+	4	3	1	4	4–	0.	3+	3	1–	0.
23	4	2	1–	1=	1	1=	1–	3+	3++	3	0;	0;
24	4	4=	0;	2=	4=	4=	4=	3++	3+	3	1=	0;
25	4	4	3+	3	1–	3	1=	3	3++	3	1=	1–
26	4	4	0;	3	4–	1=	3++	1=	4=	3	1–	1+
27	4–	2	0	0.	1=	1	1–	4=	3++	1=	4=	1+
28	4	2	0.	3	4–	1	4=	3	3	3	1=	0.
29	4	4–	0	3	x++	x±	x+	x	x+	3	1–	1–
30	4	4	0.	3++	x++	x±	x+	x	x+	3+	4=	1
31	4+	4	3++	2–	x–	x+	x±	x	x+	3+	1–	1
32	4	4=	4=	3+	x+	x±	x±	x	x+	3	1=	1–
33	4+	2	4	1+	1=	1–	1	4=	3++	3	1–	1–
34	4+	4–	4–	4=	4	4=	4=	4=	3++	1=	0;	1=
35	4	4=	3+	0;	1=	1–	1=	3+	3++	3	0;	1
36	4	4	4–	3++	1=	1=	0;	x	3++	3	0;	1–
37	4	4–	0	3++	4=	4=	4=	x	3–	3	1=	1–
38	4	2=	4–	3++	x+	x±	x+	x++	x++	4–	1=	1+
39	4–	2=	4=	3+	4+	3++	4–	4=	3++	4=	1=	1–
40	4+	4+	4	4+	4+	4+	4	4=	4	0;	4=	1=
41	2++	4	0	0;	4=	4	4+	4+	4–	4–	1–	4e
42	4	4	0	0;	4+	4	4	4	4	4=	2=	4e
43	4+	3++	0	0;	0;	1=	0;	x	1	3	0;	1–
44	4	3++	0	0;	0;	0;	0;	3+	3+	3	1	0;
45	4	2	0	2–	4	4	4	x	x	3	3	1
46	4	3++	0	2–	4	4	4	1	1	3	3	1
47	4	2–	0	1±	x–	x;	x++	x;	x±	1	0;	1
48	4+	1	0;	1+	x+	x=	x±	x+	4–	4=	1=	1+
49	4	4–	0	4=	1=	1–	0;	x=	3+	1–	0;	1=

TABLE 3—(*Continued*)

Physiologic forms	Reaction of differential varieties[a]											
	Little Club	Marquis	Kanred	Kota	Arnautka	Mindum	Spelmar	Kubanka	Acme	Einkorn	Vernal	Khapli
50	4+	2+	0;	2++	1–	0;	0;	x=	x=	0;	0;	0;
51	4	2=	3+	0;	0;	0;	0;	4	3++	3+	4–	0;
52	4	4	4–	4	1=	1=	1=	x±	4	4–	4+	1–
53	4	2±	0	1	4	4	4	4	4	3±	3±	1
54	4	3++	0	0;	0;	0;	0;	1	3+	3	1	0;
55	4	4	0	2–	4	4	4	x	x	3	3	1
56	4	3+	3+	3+	1=	1=	1=	3+	3+	1=	1=	1–
57	4	4–	0.	3+	1	1	1	4	3+	3	3	1

[a] Explanation of symbols: 0—absolute immunity, 1—extreme resistance, 2—moderate resistance, 3—moderate susceptibility, 4—complete susceptibility, x—indeterminate reaction, (;)—hypersensitive flecks, (.)—necrotic lesions, (:)—hypersensitive islands; plus and minus signs indicate a slightly greater or smaller amount of rust than the nearest figure representing the infection types; c—chlorosis, n—necrosis.

RESULTS OF THE PHYSIOLOGICAL-FORM SURVEY IN 1926

Physiologic forms isolated: their prevalence and distribution. In 1922 Stakman and Levine (13) described 37 physiologic forms of *Puccinia graminis tritici*. All of these had been found at one time or another in North America. Between 1922 and 1926 2 additional forms were identified from rust collections made in the United States, thus making a total of 39 forms known to exist in North America. In 1926, 1927, and 1928 8 additional forms were discovered in the United States and Canada.

The following 18 forms were isolated in 1926: 1, 3, 11, 14, 17, 18, 19, 21, 23, 29, 30, 32, 33, 34, 36, 38, 39, and 51. Of these, forms 18 and 36 were by far the most common. Together they comprise 65 of the 138 identifications, having been collected 37 and 38 times, respectively. Other forms, such as 30 and 19, were collected only once and twice, respectively.

The distribution and prevalence of the forms isolated by the writer in 1926 are shown in table 4 and in figure 1. They are briefly summarized as follows:

Form 1. Found twice in 1926. Collected in Nova Scotia and Ontario, Canada. Not collected in the United States.

Form 3. Found 9 times. Collected in Colorado, Montana, Ohio, and Oklahoma. Found also in Quebec and New Brunswick, Canada.

Form. 11. Most prevalent form collected in southern United States. Found 6 times in Texas, and once each in Arizona, Georgia, and Kentucky.

Form 14. Collected once in Utah and once in Ontario.

Form 17. Isolated from 9 collections. Widely distributed; found in California, Kentucky, North Carolina, Oklahoma, Texas, Utah, Quebec, and Nova Scotia.

TABLE 4.—*Prevalence and distribution of physiologic forms of Puccinia graminis tritici isolated in North America in 1926*

Distribution	Physiologic forms																		Total number	
	1	3	11	14	17	18	19	21	23	29	30	32	33	34	36	38	39	51		
	Number of times isolated																		Cultures	Forms
United States																				
Alabama																	1		1	1
Arizona			1			1											1		3	3
California					1														1	1
Colorado		1				2		1							1				5	4
Georgia			1																1	1
Indiana						2				1									3	2
Iowa						2													2	1
Kansas						1		1						1	2				5	4
Kentucky			1		1			1									1		4	4
Michigan						1		2							1				4	3
Minnesota		1				10		1				2	1		5	1		1	22	8
Missouri						1													1	1
Montana		1				2									1				4	3
Nebraska						2		2							4				8	3
North Carolina					1														1	1
North Dakota						2		2							2				6	3
Ohio		1				1									2	1			5	4
Oklahoma		1			1														2	2
Pennsylvania									1										1	1
South Dakota						2						1			1				4	3
Texas			6		1	1	1	2							2		4		17	7
Utah				1	1	1					1	1			1				6	6
Virginia									1										1	1
Wisconsin						2						1			2				5	3
Wyoming						3						1							4	2
Canada																				
Quebec		3			2		1			1		3			3				13	6
New Brunswick		1													1				2	2
Nova Scotia	1				1	1													3	3
Ontario	1			1								2							4	3
Number of times each form was found	2	9	9	2	9	37	2	12	2	2	1	11	1	1	28	2	7	1	138	18

Form 18. The most prevalent form in 1926. It was wide-spread; found in Utah, Arizona, Texas, and Nova Scotia, as well as rather generally distributed over intermediate areas. Isolated 37 times during the season.

Form 19. Found twice. Collected in Texas and Quebec.

Form 21. Third in prevalence among the forms isolated. Found 12 times; quite generally distributed. Collected in Colorado, Kansas, Kentucky, Michigan, Minnesota, Nebraska, North Dakota, and Texas.

Form 23. Found only twice during the season. Collected in Pennsylvania and Virginia.

Form 29. Found once in Indiana and once in Quebec.

Form 30. One collection of form 30, late in the season at Fort Duchesne, Utah.

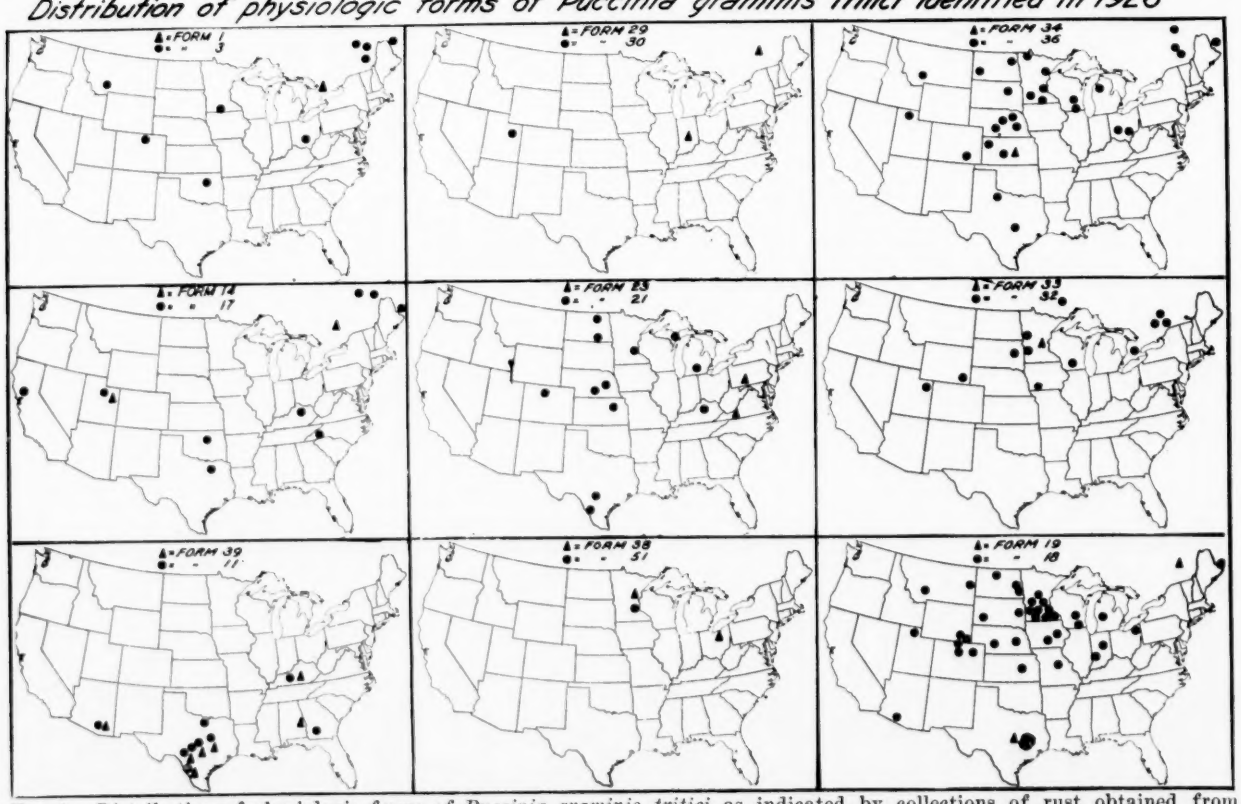

FIG. 1. Distribution of physiologic forms of *Puccinia graminis tritici* as indicated by collections of rust obtained from different States in the United States and from Canada and identified in 1926.

Form 32. Isolated from 11 collections. Found in Minnesota, South Dakota, Utah, Wisconsin, and Wyoming, in the United States, and in Ontario and Quebec, in Canada.

Form 33. Collected once in Minnesota.

Form 34. Found once in Kansas.

Form 36. Found 28 times; distributed over practically the same area as form 18. Collected in 12 States of the United States and in 2 Provinces of Canada.

Form 38. Found twice, once in Minnesota and once in Ohio.

Form 39. Apparently restricted to the South. Distribution much the same as that of form 11. Collected 4 times in Texas and once, each, in Arizona, Alabama, and Kentucky.

Form 51. Collected once in St. Paul, Minn.

Identity and nature of isolated physiologic forms. The reactions of the differential varieties in the greenhouse to the forms isolated in 1926 are as follows. Of the *vulgare* wheats Marquis was susceptible to 11 of the 18 forms. Kanred was susceptible to 10, highly resistant to 4, and immune from the remaining 4 forms. Kota was susceptible to 14 of the 18 forms.

Of the durum wheats, Arnautka, Mindum, and Spelmar reacted the same. They were susceptible to 7 forms, resistant to 7, and reacted heterogeneously[5] to 4 forms. Kubanka was susceptible to 12 forms and resistant to 1 and gave a heterogeneous reaction to 5. Acme was susceptible to 14 forms and reacted heterogeneously to 4.

Little Club, the only *compactum* wheat in the list of differentials, was susceptible to all forms isolated, while Einkorn, C. I. 2433, the only representative of *Triticum monococcum*, was susceptible to 17 of the 18 forms.

Of the emmers, Khapli was resistant to all of the 18 forms and Vernal was resistant to all but 2.

Evidence of migration of rust from South to North: (Comparison of southern and northern physiologic forms.) A comparative study of the southern and northern physiologic forms in 1926 suggests that there was no general migration of stem rust from South to North.

This year form 11 was the most common form found in Texas. It was found also in Arizona, Georgia, and Kentucky but was not identified in the Minnesota laboratory from any other States. Six of the 17 collections from Texas were form 11. Although this form was not identified by the writer from either the hard-red-winter or hard-red-spring-wheat regions, Peltier and Thiel (11) report having collected it 13 times in 1926. The majority of these 13 collections were made in Nebraska, but these authors mention that they identified form 11 also from collections made in Arizona and

[5] On a single leaf there may be a range of infection from necrotic flecks to type-4 pustules not subject to mechanical separation.

Texas. They found form 11 on wild grasses near infected barberries and also in grain fields where the infection could not be traced to barberries. Peltier and Thiel (11) are of the opinion that form 11 originated on barberries in northeastern Nebraska and that possibly some inoculum of this form may have come from other sources out of the State.

Stakman, Levine, and Wallace (16) suggest that Peltier and Thiel may have, in some instances at least, identified form 32 as form 11. Form 32 is identical with form 11 except that it produces a heterogeneous or type-x infection on the durum varieties, whereas form 11 produces a type-4 infection. Under certain environmental conditions the type-x infection may appear as type-4, thus causing the collection to be identified as form 11. It can not be said with certainty that this actually occurred in the case of the collections of Peltier and Thiel, but that such may have happened is suggested by the statement of these authors regarding the differences in degree of virulence of different collections of form 11. They found collections of this form to be outstandingly different in virulence. To the writer, this suggests that Peltier and Thiel were dealing with a form that produces the heterogeneous, or type-x, infection on the durum varieties, very likely form 32.

Form 39 seemed rather closely associated in 1926 with form 11, as these 2 forms were found together in 4 locations in Texas and also at Sacaton, Ariz., and Lexington, Ky. It was found also in Alabama but was not collected in the States north of Texas.

Form 18[6] was found in Texas, at College Station, and form 36 was found at Georgetown and Chillicothe, Tex. These forms were the 2 most common in the northern collections. Form 21 was found twice in Texas and was found later in 10 other collections from Nebraska, Kansas, Kentucky, Colorado, North Dakota, Minnesota, and Michigan. Since forms 18, 21, and 36 were found to be those most common in collections from the northern wheat fields, and, since all 3 of them were known to be present in Texas, it might seem that these forms may have migrated from Texas. However, further study of the prevalence and distribution of forms other

[6] Some doubt has recently developed as to the differentiation between form 18 and form 36. Their behavior is identical on all differential varieties with the exception of Kubanka. Form 18 produces a 4-type infection on Kubanka, indicating complete susceptibility. Form 36 produces the heterogeneous, or type-x, infection. Under certain environmental conditions form 36 may produce what appears to be a type-4 infection, the flecks and small pustules of the type-x apparently being masked by the large pustules. In such instances, if additional inoculations are not made on Kubanka, the culture might be identified as form 18. It is believed that form 18 actually exists, yet it is interesting to know that at the Dominion Rust Research Laboratory of Canada form 18 was not identified at all between 1923 and 1928, while over 500 collections were identified as form 36. In this paper forms 18 and 36 are discussed as 2 distinct forms.

than 18, 21, and 36 does not support the assumption that there was a general migration of stem rust from the South to the North.

Since forms 11 and 39 were more prevalent in Texas wheat fields than other forms and were not found elsewhere in the central wheat region, except possibly form 11 in Nebraska, it is difficult to explain how forms 18, 21, and 36 could have been blown northward from Texas, leaving forms 11 and 39 behind. This, likewise, applies to form 19, which was found in Texas but at no other place in the United States.

There is additional evidence that form 21 did not migrate northward from Texas. Forms 21 and 11, respectively, were collected in the same field at Laredo, Tex. At San Antonio form 21 was collected, and form 11 was found at Castroville, only a few miles from San Antonio. If form 21 migrated northward from these locations in Texas it seems reasonable to assume that form 11 also would have migrated because it has a wider host range than form 21. It attacks Kanred wheat, which is immune from form 21, and considerable Kanred wheat is grown in the hard-red-winter-wheat region.

Form 17 was collected at Denton, in northern Texas, and form 11 was isolated from the same collection. Form 17 was collected later at Stillwater, Okla., along with form 3. Form 11 was not found in Oklahoma and form 3 was not found at all in Texas. However, form 11 might have been present in Oklahoma, as only 1 rust collection was identified from that State. Apparently, form 17 did not spread from either Texas or Oklahoma to the more northern regions of the Mississippi Valley. It was found in North Carolina, Utah, California, Kentucky, and eastern Canada, Oklahoma, and Texas, but not elsewhere.

Form 32 ranked fourth in prevalence in 1926. It was collected 10 times and was present in Utah, Wyoming, South Dakota, Minnesota, and Wisconsin, and in Canada. This form was not collected in the southern United States.

Since a rather severe epiphytotic of stem rust occurred in Arizona and southern California in 1926, this area naturally has been considered as a possible source of inoculum.

Only 1 collection of stem rust was obtained from Arizona, from which were isolated forms 11, 18, and 39. Nine collections were made in northeastern Colorado and southeastern Wyoming. Five of these were form 18. Forms 11 and 39 were not collected in Colorado or Wyoming.

Comparison of United States survey with Canadian survey. Data on the survey conducted in 1926 by the Dominion Rust Research Laboratory at Winnipeg, have recently become available (7, 8) and it is of interest to note these results. The results of the Canadian survey are compared with those of the United States in table 5.

Fifteen forms were isolated by the Canadian workers in 1926. Ten of these were found by the writer in 1926. The other 5 forms, isolated in the Canadian survey and not found by the writer, comprise collectively only a total of 9 collections. Three of these forms, namely, 48, 49, and 52, had never been identified before. The writer isolated 8 forms that were not identified in the Canadian laboratory. These were 1, 3, 11, 18, 23, 33, 39, and 51. Forms 1 and 3 were isolated from Canadian collections. Of the 13 forms not isolated by both laboratories, probably only 3 were common enough to be of any significance in studying rust migration. Form 3 was not found in the Canadian survey, but it occurred in Canada, since the writer isolated it from 4 rust collections from Quebec and New Brunswick. It is interesting to note that forms 11 and 39 were not identified from any

TABLE 5.—*Summary of the prevalence of physiologic forms of Puccinia graminis tritici in 1926 as shown by the surveys of the United States Department of Agriculture and of the Dominion Rust Research Laboratory of Canada*

Physiologic forms	Number of times isolated		
	United States	Canada	Both surveys
1	2	...	2
3	9	...	9
9	...	1	1
11	9	...	9
14	2	2	4
15	...	2	2
17	9	2	11
18	37	...	37
19	2	1	3
21	12	86	98
23	2	...	2
29	2	29	31
30	1	7	8
32	11	12	23
33	1	...	1
34	1	4	5
36	28	217	245
38	2	18	20
39	7	...	7
48	...	2	2
49	...	3	3
51	1	...	1
52	...	1	1
Total number of isolations	138	387	525
Total number of forms isolated	18	15	23

Canadian collections. It seems safe to conclude that had forms 11 and 39 migrated northward from Texas they would have been isolated at least a few times from the approximately 500 collections made in the northern United States and Canada.

In Canada form 36 was most prevalent by far, but it should be remembered that all collections of form 18, if there is such a form, are listed by the Canadian workers as form 36. Form 21 ranked second in Canada and this is in agreement with the United States survey. Form 29 ranked third and this form was found only once in the United States. It appears that form 29 certainly must have originated on barberries. Whether these were local barberries or not is undetermined.

PHYSIOLOGICAL FORMS EXPECTED FROM BARBERRIES IN 1926

If it were possible to collect and identify a large number of aecial infections on barberries each year and then correlate the physiologic forms on barberries with the forms occurring in the fields, both in the barberry region and in the South, the source of inoculum could be rather definitely established. For instance, if the predominant forms found on barberries are the same as those occurring on wheat and grasses in the spring-wheat area but different from the southern forms, then it would be quite certain that the barberries were responsible for the stem rust occurring that year. In a similar way it could be shown that both the barberries and the southern wind-blown inoculum contributed towards an epiphytotic or that only the southern source was of importance.

Since the 1926 survey was well advanced before the writer outlined this particular problem, no aecial collections were obtained. In 1927 and 1928 an attempt was made to obtain aecial material and to make identifications of such collections The results were quite disappointing, however, as no satisfactory method of obtaining fresh, viable aecia was devised. In 1928, over 200 aecial collections were obtained, but relatively few produced infection after being transferred to the proper hosts.

It would be expected that physiologic forms common one year would be likewise common the next year through the agency of barberries, but with the many factors, such as teliospore viability and germination, infection of barberries, and recent evidence of the origin of new forms through hybridization (10, 19) influencing the behavior of stem rust, there may be no regular correlation between the occurrence of certain forms one year and their occurrence the following year. The writer, as well as others,[7] has noticed the extreme scarcity or even absence of collections of certain forms in one season even though these same forms were quite common the previous season. That certain forms have been rather regular in their occurrence

[7] Stakman and Levine—unpublished data.

and then apparently disappear for 1, 2, or more years, is a well-established fact, as far as the annual surveys demonstrate, but an explanation for such behavior is still lacking. More extensive surveys with more carefully planned field collections might reveal the presence of every known form each year. However, it seems that the collections, though limited in number, should be indicative of the relative prevalence of those forms to which the commonly grown wheat varieties are susceptible.

The 4 most commonly isolated forms in the United States in 1926 were 18, 36, 21, and 32. The survey records show that forms 18, 32, 21, and 11 were the most prevalent in 1925. The scarcity of form 36 in 1925 could possibly be explained by the fact that many collections may have been identified as form 18 that in reality were form 36. Form 11 ranked fourth in 1925 but was collected only 9 times and that over a wide area.

In 1925, in Canada, only 3 forms were of very much importance, according to the survey conducted at the Dominion Rust Research Laboratory. They were, in order of their prevalence, forms 36, 21, and 29. In 1926 these three forms again predominated and their increased numbers of isolations in 1926 were almost exactly proportional to the increase of the total collections of 1926 over those of 1925. In 1926 form 38 ranked fourth in Canada. This form had never been identified there previously. This is probably explained by the fact that until 1926 the Canadian survey included only the western Provinces, *i.e.*, Manitoba, Saskatchewan, and Alberta. In 1926 the eastern Provinces were included in the survey and form 38 was collected in that region 17 times, whereas it was collected only once in the western Provinces. Newton, Johnson, and Brown (9) describe form 38 as characteristically an eastern form. The writer finds this to be somewhat the case as shown by surveys conducted at the University of Minnesota. Since Marquis is resistant to form 38, Newton, Johnson, and Brown attribute its prevalence in eastern Canada to the growing of many different wheat varieties, most of which are susceptible to form 38. They conclude that its gradual spread westward has been influenced by the introduction of susceptible varieties. Mention has been made of form 38 here because of the fact that it was again rather common in 1927, as will be brought out later in this paper.

The rather common occurrence, in 1926, of certain physiologic forms that had been the most common forms in 1925, as shown by both the United States and Canadian surveys, suggests that the northern rust in 1926, probably, for the greater part, originated on barberrries.

RESULTS OF THE PHYSIOLOGIC-FORM SURVEY IN 1927

Physiologic forms isolated: their prevalence and distribution. In 1927, the following 19 physiologic forms were isolated: 1, 3, 11, 14, 17, 18, 19,

21, 23, 28, 30, 32, 33, 36, 38, 39, 49, 50, and 52. Form 52 had never before been isolated in the United States, but it had been found in 1926 by the Canadian workers at the Dominion Rust Research Laboratory, at Winnipeg. Peltier and Thiel (11) collected a new form 4 times in Nebraska in 1926, which was later given the number 49. Four hundred and ten identifications were made in 1927. As in 1926, form 18 was the most prevalent of the forms identified and form 21 was a close second. Forms 39 and 36 ranked third and fourth, respectively.

The prevalence and distribution of those forms isolated in 1927 are given in table 7 and figure 2 and are summarized as follows:

Form 1. Isolated 8 times; found in Texas, Oklahoma, Kansas, Illinois, Idaho, Wisconsin, and Pennsylvania.

Form 3. Found once in Montana.

Form 11. Isolated from 17 collections; found in Texas 7 times, Kansas 3 times, Ohio twice, and once, each, in Tennessee, North Dakota, Missouri, Indiana, and Iowa.

Form 14. Found once in Montana and twice in California.

Form 17. Isolated from 17 collections. Found in Texas, Oklahoma, Nebraska, South Dakota, Missouri, Minnesota, Montana, and Pennsylvania.

Form 18. Extremely common and wide-spread. Found in 21 of the 25 States included in the survey. Isolated from 124 collections.

Form 19. Found 8 times. Twice, each, in Texas and Kansas and once, each, in Minnesota, Montana, Nebraska, and Pennsylvania.

Form 21. Found 120 times; widely distributed, collected in 21 States. Its prevalence and distribution were essentially the same as those of form 18.

Form 23. Found twice only, both collections from Pennsylvania.

Form 28. Isolated once in Texas; not found elsewhere.

Form 30. Collected once in Illinois.

Form 32. Found 14 times; quite widely distributed.

Form 33. Found once, each, in Wisconsin, Michigan, and Ohio.

Form 36. Isolated 29 times from collections in 14 different States.

Form 38. Collected 18 times from 10 States. Rather common in collections from Eastern States.

Form 39. This form ranked third in prevalence, being isolated 33 times; rather generally distributed; collections made in 15 States.

Form 49. Although form 49 had never been found in the United States previous to 1927, except in Nebraska, it was collected 9 times in the United States in 1927, from 8 States.

Form 50. Collected for the first time in the United States in Michigan.

Form 52. Found for the first time in the United States; collected only once, in Arizona. This form was found once in 1926 by the Canadian investigators.

TABLE 6.—*Prevalence and distribution of physiologic forms of Puccinia graminis tritici isolated in North America in 1927*

Distribution	Physiologic forms																		Total number	
	1	3	11	14	17	18	19	21	23	28	30	32	33	36	38	39	49	50	52	
	Number of times isolated																		Cultures	Forms
United States																				
Arizona	...	...	...	...	...	2	...	1	...	...	...	2	...	...	...	...	...	...	1	6	4
California	...	...	...	2	...	2	...	...	...	...	...	...	...	...	...	1	...	...	...	5	3
Colorado	...	...	...	...	...	5	...	3	...	...	...	...	...	1	...	...	...	...	...	9	3
Georgia	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	1
Idaho	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1
Illinois	1	...	...	...	...	...	...	1	...	...	1	1	...	1	...	1	...	...	...	6	6
Indiana	...	...	1	...	...	2	...	1	...	...	...	...	...	...	...	2	...	...	...	6	4
Iowa	...	...	1	...	...	2	...	6	...	...	...	...	...	3	1	...	...	...	...	13	5
Kansas	1	...	3	...	...	18	2	9	...	...	...	1	...	1	...	7	1	...	...	43	9
Kentucky	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	1	...	...	...	2	2
Michigan	...	...	...	...	...	1	...	2	...	...	...	1	1	...	...	...	1	1	...	7	6
Minnesota	...	...	...	...	2	15	1	10	...	...	...	3	...	6	...	1	2	...	...	40	8
Mississippi	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1
Missouri	...	...	1	...	1	7	...	6	...	...	...	...	...	...	...	2	1	...	...	18	6
Montana	...	1	...	1	1	7	1	2	...	...	...	2	...	1	...	...	...	...	...	16	8
Nebraska	...	...	...	...	3	6	1	5	...	...	...	...	...	3	...	1	1	...	...	20	7
New York	...	...	...	...	...	2	...	1	...	...	...	...	...	...	1	...	...	...	...	4	3
North Dakota	...	...	1	...	...	14	...	16	...	...	...	...	...	2	...	...	...	...	...	33	4
Ohio	...	...	2	...	...	2	...	2	...	...	...	2	1	4	6	7	1	...	...	27	9
Oklahoma	2	...	...	...	1	8	...	10	...	...	...	1	...	2	1	2	1	...	...	28	9
Pennsylvania	1	...	...	...	2	...	1	...	2	...	...	...	...	...	1	1	...	...	...	8	6
South Dakota	...	...	...	...	4	9	...	14	...	...	...	1	...	1	1	2	...	...	...	32	7
Tennessee	...	...	1	...	...	1	...	1	...	...	...	...	...	...	1	1	...	...	...	5	5
Texas	1	...	7	...	3	12	2	20	...	1	...	...	...	2	3	1	...	...	...	52	10
Virginia	...	...	...	...	...	1	...	2	...	...	...	...	...	...	2	3	...	...	...	8	4
Wisconsin	1	...	...	...	...	4	...	6	...	...	...	...	1	1	...	...	1	...	...	14	6
Wyoming	...	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	1	2
Canada																				
Saskatchewan	...	...	...	...	...	3	...	1	...	...	...	...	...	...	...	...	...	...	...	4	2
Number of times each form was found	8	1	17	3	17	124	8	120	2	1	1	14	3	29	18	33	9	1	1	410	19

Identity and nature of isolated physiologic forms. The reactions of the 12 differential varieties to each of the physiologic forms isolated in 1927 are as follows. Little Club was susceptible to all the 1927 forms. Marquis was resistant to 8, Kanred to 10, and Kota to 4 of the 19 forms isolated. The durum varieties, Arnautka, Mindum, and Spelmar, reacting as a group, were resistant to 9 of the forms and responded heterogeneously to 3 others, producing the type-x infection. One form was collected to which Mindum was resistant, while all other durum varieties were susceptible. Kubanka was resistant to only 1 form and reacted heterogeneously to 7 forms. Acme was susceptible to 15 forms and reacted heterogeneously to the other 4.

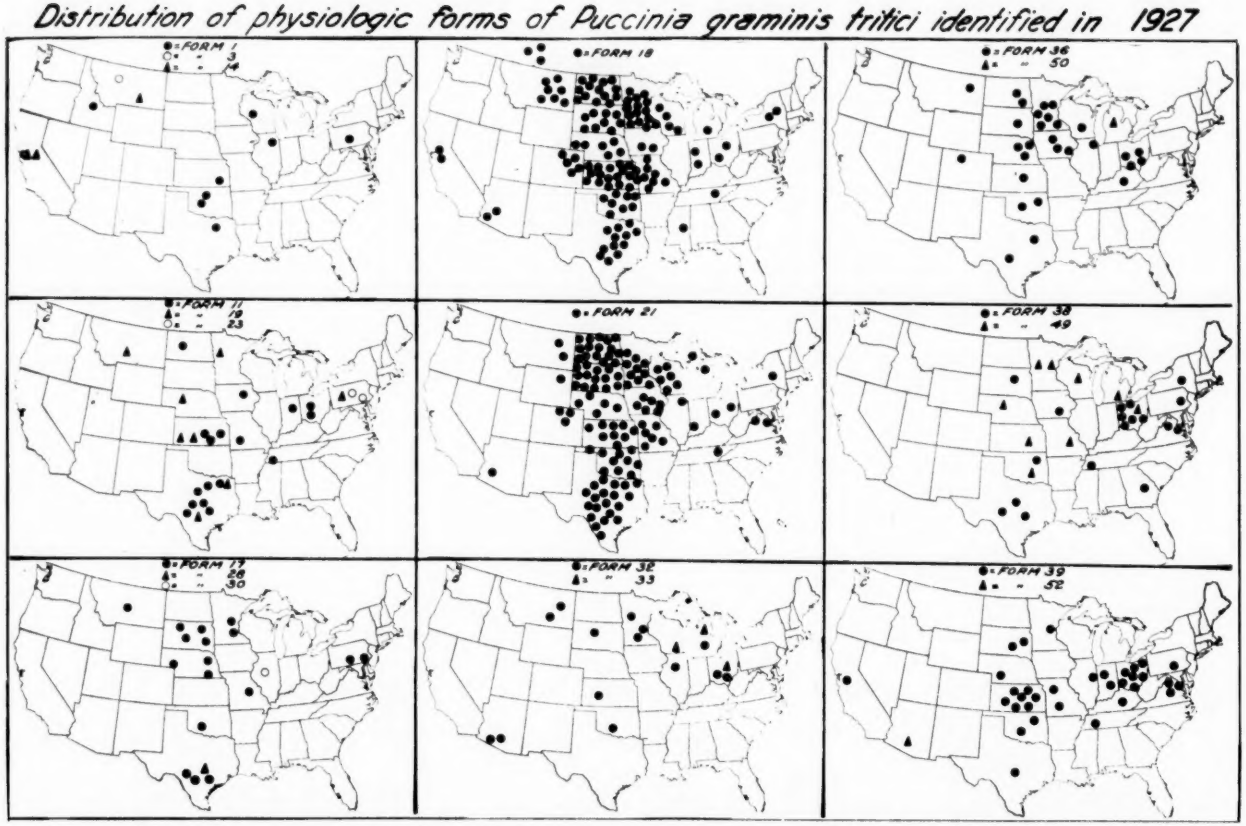

FIG. 2. Distribution of physiologic forms of *Puccinia graminis tritici* as indicated by collections of rust obtained from different States in the United States and from Canada and identified in 1927.

Einkorn was resistant to 3 forms. Only 2 forms were found that attacked Vernal heavily, namely, forms 30 and 52, each of which was found only once. None of the 19 forms isolated was capable of producing more than flecks and very minute type-1 pustules on Khapli.

Evidence of migration of rust from south to north: Comparison of southern and northern physiologic forms. In 1927, 410 identifications were made by the writer from rust collections in North America. Of these, 406 were made from rust collected in the United States. The places of collection were more or less uniformly distributed, especially over the principal wheat-growing States of the Mississippi Valley. Fifty-two identifications were made from Texas collections, 28 from Oklahoma, 43 from Kansas, 20 from Nebraska, 18 from Missouri, and the hard-red-spring-wheat States were, likewise, fairly well covered. More collections than were obtained from the Great Lakes States would have been desirable in order to more accurately compare that region with Texas as a source of inoculum for the hard-red-spring-wheat States. However, there were 60 identifications made from Wisconsin, Illinois, Indiana, Michigan, and Ohio collections, as compared to 52 identifications from Texas collections. If aeciospores produced on barberry bushes in the Great Lakes States were responsible for any of the rust that developed later in the wheat fields of Minnesota and the Dakotas, these same barberry bushes would be expected to be responsible for a considerable part of the field infection in the Great Lakes States. Even though the numbers were relatively small, it seems that random field collections would yield the forms that had become best established in a particular region. Forms to which most of the commonly grown wheat varieties are resistant naturally stand less chance of being collected.

The larger number of identified collections in 1927 over those of 1926 gave much better data for studying the possible migration of rust from South to North. From the available data, it does not appear to the writer that the Great Lakes States were so important as a source of inoculum in 1927 as was Texas. There was a total of 72 identifications made from Illinois, Indiana, Michigan, Ohio, Pennsylvania, New York, and Wisconsin. Thirty-three per cent of these were forms 18 and 21, whereas these 2 forms comprised a total of 73 per cent of the 52 identifications from Texas. Wisconsin is so located geographically that it could have received inoculum from both sources, so that it may be fairer to omit it from the Great Lakes States group. Without the Wisconsin collections forms 18 and 21 would comprise 24 per cent of the total of 58 identifications from the other Great Lakes States.

It is of further interest to note that in some of the Great Lakes States there was no outstanding form, so far as the collections showed. Six identifications from Illinois produced 6 different forms; 4 from Indiana, 3 forms; 7 from Michigan, 6 forms; 4 from New York, 3 forms; 8 from Penn-

sylvania, 6 forms. Ohio was an exception to this, as there were 9 forms from 27 identifications, forms 36, 38, and 39 being collected more often than any others. In Minnesota there were 8 forms in 40 identifications; in North Dakota there were only 4 forms in 33 identifications; and in South Dakota 7 forms were found in 32 identifications. In a total of 105 identifications from the last 3 named States there were only 10 forms. Fifteen forms were isolated from 72 cultures of rust from the Great Lakes States.

The above data suggested that in the barberry area there may occur a greater variety of forms than in more distant areas. Theoretically, this should be expected.

Form 38 was found 6 times in Ohio and form 39 was found 7 times. Form 38 was found once in New York and once in Pennsylvania, while form 39 was found once in Illinois, once in Pennsylvania, and twice in Indiana. Both forms 38 and 39 were rather scarce in the collections from the hard-red-spring-wheat States, being found only once and 3 times, respectively, in the whole of Wisconsin, Minnesota, North Dakota, South Dakota, and Montana.

Forms 1, 23, 30, 33, and 50 were collected in the Great Lakes States in 1927 but were not recovered from any of the collections in Minnesota, North Dakota, South Dakota, and Montana. Marquis and some of the durum wheats are resistant to forms 23, 33, and 50, but forms 1 and 30 should have the same chance of finding susceptible varieties as forms 18 and 21.

In 1927 forms 18 and 21 were predominant, having been collected 124 and 120 times, respectively. Their prevalence and regular distribution are quite striking, as shown by the maps in figure 2. Beginning with Texas, where these 2 forms were found 12 and 20 times, respectively, and, moving northward, they were collected as follows: Oklahoma, 8 and 10; Kansas, 18 and 9; Nebraska, 6 and 5; Missouri, 7 and 6; South Dakota, 9 and 14; North Dakota, 14 and 16; Montana, 7 and 2; Minnesota, 15 and 10; and Wisconsin, 4 and 6. The regularity with which these 2 forms were collected throughout the season, after having been outstandingly common in Texas in the spring, suggest a possible dissemination of inoculum from Texas to the Northern States.

Additional evidence that there was an appreciable migration of stem rust from the south in 1927 lies in the fact that of the 10 most prevalent forms over the entire surveyed area 9 were present in Texas. Form 32 was found 14 times but was not collected in Texas. Form 28 was collected once in Texas but not elsewhere.[8] Of the 19 forms isolated in 1927, only 10 forms were found more than 3 times, each. With the exception of form 32, all physiologic forms found more than 3 times, each, during the year also were collected in the same season in Texas.

[8] Marquis, Mindum, and Kanred varieties of wheat are highly resistant to this form. This may decrease the possibility of collecting it in the hard-red-spring- and durum-wheat fields. It never has been a common form, so far as the surveys indicate.

COMPARISON OF UNITED STATES SURVEY WITH CANADIAN SURVEY

A summary of the results obtained by the Dominion Rust Research Laboratory survey of 1927 and of the United States Department of Agriculture survey of the same year is given in table 7. At Winnipeg 511 identifications were made and 19 physiologic forms were isolated. The 2 most prevalent forms in the United States in 1927 were 18 and 21, whereas in Canada they were forms 36 and 21. It should be remembered that at the Canadian laboratory form 18 is not distinguished from form 36. If forms 18 and 36 were combined in the United States as is done in Canada, there would have been 153 collections of form 36. Then, with 120 collections of form 21, forms 21 and 36 would comprise 273 of the 410 identifications made, or about 67 per cent. In Canada these 2 forms comprised 369 of the 511 identifications, or approximately 72 per cent. These data certainly seem to indicate that most of the stem rust of wheat in Canada in 1927 originally came from the same source as that in the Mississippi Valley of the United States. It seems also to support the belief that in 1927 there was a migration of stem rust of wheat from South to North. It is quite clear that the southern wheat fields are not the only source of inoculum for Canadian fields It is the writer's contention that under favorable climatic conditions the barberry is always responsible for a certain amount of stem rust but that severe and widespread epiphytotics probably result only when both sources supply inoculum and when proper environmental factors are present for the development and dissemination of urediniospores. That western Canada receives inoculum from other sources than the adjoining States of the United States is brought out by the fact that each year certain forms that are not found at all in the United States occur rather commonly in Canada. In the Prairie Provinces, Manitoba, Saskatchewan, and Alberta, form 15 was collected 9 times in 1927. This form was not collected in the United States a single time. In this same region form 14 was collected 15 times. It was collected only once in the United States, in Utah. Likewise, forms 16, 29, 34, 56, and 27 were collected 2, 5, 12, 1, and 1 times, respectively, in western Canada and were not found in the United States at all. Form 52 was collected 13 times in Manitoba and Saskatchewan and was found only once in the United States (Arizona), the only time it has ever yet been found in the United States. It would seem that the lateness of the season in Canada would permit the accumulation of rust forms from many different regions.

Physiologic forms of stem rust of wheat expected from barberries in 1927. Since the eastern United States was not covered sufficiently by the survey of 1926, it is hardly worth while to speculate as to the forms that might be expected on barberries there in 1927. In the Middle West, the only forms that would be expected in 1927, because of their abundance in

TABLE 7.—*Summary of the prevalence of physiologic forms of Puccinia graminis tritici in 1927 as shown by the surveys of the United States Department of Agriculture and of the Dominion Rust Research Laboratory of Canada*

Physiologic forms	Number of times isolated		
	United States	Canada	Both surveys
1	8	.....	8
3	1	.....	1
9	.....	7	7
11	17	.....	17
14	3	16	19
15	.....	9	9
16	.....	2	2
17	17	18	35
18	124	.....	124
19	8	1	9
21	120	141	261
23	2	.....	2
28	1	.....	1
29	.....	5	5
30	1	10	11
32	14	7	21
33	3	.....	3
34	.....	12	12
36	29	228	257
38	18	25	43
39	33	.....	33
49	9	10	19
50	1	4	5
52	1	13	14
53	.....	1	1
56	.....	1	1
57	.....	1	1
Total number of isolations	410	511	921
Total number of forms isolated	19	19	27

1926, were forms 18, 21, 36, and possibly 32. The first 3 forms were very common in 1927 and no doubt these forms were disseminated to some extent by barberries. However, as discussed previously, it appears that part of the damage done by these 3 forms in 1927 was due to their abundance in southern fields early in the season and their consequent migration northward. Form 32 evidently did not come from Texas in 1927, and it was not found there at all. Apparently, it came principally from barberries, although it was obtained once from the 28 collections made in Oklahoma.

It was not common anywhere, being found only 14 times, a small number as compared with the collections of forms 18 and 21.

On the other hand, certain forms were common in 1927 in the barberry area of the northern United States that were not found there in sufficient quantity in 1926 to lead one to expect them to occur on barberries in 1927. Forms 11 and 39 were not found at all in the Mississippi Valley north of Texas in 1926. These 2 forms were found 10 and 32 times, respectively, outside of Texas in 1927. Form 11 could easily have migrated from Texas, since it was collected there 7 times. Form 39 was found only once in Texas, twice in Oklahoma, and 7 times in Kansas. That spores of this form developed in Texas and migrated northward, increasing in number on the way, is possible; but, since it was rather common in Ohio and was found also in Virginia, Kentucky, Pennsylvania, Indiana, and Illinois of the States east of the Mississippi River, it is possible that it may have come principally from eastern barberries.

Form 17 was not found in the Mississippi Valley north of Oklahoma in 1926 and therefore it was not expected from barberries in the spring-wheat area in 1927. This form was found 3 times in Texas and 11 times in the other valley States in 1927. This would suggest Texas as its starting place.

The Canadian survey of 1926 showed forms 21, 29, 32, 36, and 38 to be present in sufficient amounts to make it reasonable to expect their recurrence the following year. Forms 29, 32, and 38 were not nearly so prevalent in 1926 as forms 21 and 36, since they were collected only 29, 12, and 18 times, respectively, as compared to 86 collections of form 21 and 217 collections of form 36. In 1927 forms 21 and 36 were collected 141 and 228 times, whereas forms 29, 32, and 38 were collected 5, 7, and 25 times, respectively. Form 29 probably did not migrate into Canada from the United States in 1927, because it was not found at all that year in the States. Form 32 was present in the States but did not seem to be very abundant in any particular locality. Form 38 was somewhat more prevalent in the States and was fairly common in Kansas and Ohio. It is probably unsafe to conclude that these last 3 forms migrated from the United States into Canada, even though the increase in number of collections of form 38 in 1927 over the number of collections of this form in 1926 in the United States and a corresponding increase in Canada suggest a migration of this form during the season. This evidence is strengthened somewhat by the fact that in 1926 form 38 was collected only once in the 3 western Provinces of Canada and was collected 12 times in these Provinces in 1927.

That certain forms evidently must persist from year to year on barberries in Canada is suggested by the results of the Canadian and United States surveys (7, 8, 9, and unpublished data of Stakman and Levine). Mention is here made of some of the evidence supporting this theory, and a more careful study of the above data would no doubt bring out

more of such evidence. From 1918 to 1927 form 30 was collected in the United States only 9 times and has been found but twice since 1922. The Canadian survey shows that form 30 was collected 7 times in 1926 and 10 times in 1927. Over this same period of 9 years form 34 has been identified at the St. Paul laboratory only 5 times from collections made in the United States. In Canada form 34 was collected 4 times in 1926 and 12 times in 1927. Why these forms are not better established in the United States is not known. A study of such forms might possibly bring out some interesting physiologic differences, such as response to environmental factors, etc., which might explain their rather capricious behavior.

RESULTS OF THE PHYSIOLOGIC-FORM SURVEY IN 1928

Physiologic forms isolated: their prevalence and distribution. In 1928, 401 identifications were made from collections of stem rust of wheat. An attempt was made to have at least 1 collection from every county in each State. The Mississippi Valley States were covered very thoroughly, but, owing to the hot weather during the summer months, the identification of physiologic forms in the greenhouse was considerably retarded and it was impossible to study all collections that were selected for identification. Approximately 400 collections were placed in cold storage in the hope that the spores would remain viable until inoculations could be made, but no infection was obtained from most of the stored collections when inoculations were made in October and November.

The 1928 survey included 61 identifications of rust collections made in Mexico.[9] No collections had been obtained from Mexico since 1923.

Nineteen different physiologic forms were isolated in 1928. Their prevalence and distribution by States are shown in table 8 and figure 3. In 1928 form 38 was collected most frequently, being identified 105 times. Form 36 was a close second; it was collected 96 times. Forms 21 and 49 ranked third and fourth, being collected 67 and 29 times, respectively. The summary of the prevalence and distribution of the forms is given below.

Form 1. Collected 8 times. Not found farther south than Kansas.

Form 2. Collected once in Minnesota and once in Wisconsin.

Form 11. Found 9 times during the season; once in Mexico, twice in Texas, once in Michigan, and 5 times in Kansas.

Form 17. Collected 14 times. Five of these collections came from Mexico. The remaining 9 collections were made in several of the central and northern Mississippi Valley States and in Arizona.

Form 18. Form 18 was studied carefully during this survey and any collections showing the least tendency towards a heterogeneous, or type-x infection on Kubanka were identified as form 36. Therefore, only 9 collec-

[9] Collections made by Mr. H. H. Thornberry, who was detailed by the U. S. Dept. of Agriculture to study rust conditions in Mexico in 1928.

tions were identified as form 18. However, these 9 collections were not continued long enough to determine whether they would constantly react as form 18. It is entirely possible that had they been cultured repeatedly they eventually would have produced type-x infection on Kubanka, which would place them with form 36. The 1928 data, obtained through a close observation of the collections of these 2 very similar forms, suggest that many of the collections identified in previous years as form 18 might have been in reality form 36. However, from a practical standpoint, the differentiation of these 2 forms is of no significance at present.

Form 19. Collected 10 times; rather widely distributed but, apparently, not predominant in any particular locality.

Form 21. Rather generally distributed and fairly common. Collected twice in Mexico, 7 times in Texas, and 57 times in the other States of the United States. One collection made in Canada.

Form 23. Collected only in Mexico. It was found 3 times.

Form 29. Found only 4 times; twice in Iowa and once each in Texas and Minnesota.

Form 30. Isolated once, from a collection made in Wyoming.

Form 32. Collected 14 times. Found once in Mexico and in 8 States of the United States, though not very common in any particular locality.

Form 34. Collected once in South Dakota.

Form 36. Collected 96 times. Found once in Mexico and 5 times in Texas. The most common form found in Iowa, Kansas, Minnesota, Missouri, North Dakota, South Dakota, and Wisconsin.

Form 38. Of the 61 identifications from Mexico, 39 were form 38. This form was found 20 times in Texas out of a total of 42 isolations made from that State. It was also the most common form found in Oklahoma and Indiana and was found in 12 other States.

Form 39. Found twice in Mexico but not in Texas. Collected 20 times in the United States; apparently, most prevalent in Iowa, Kansas, and Nebraska.

Form 49. Collected 29 times. Four of these collections were from Mexico. Found 4 times, each, in Texas and Oklahoma and present in 10 other States.

Form 50. Found once in Mexico but not in the United States.

Form 52. Collected once in Iowa, once in Minnesota, once in California, and once in Mexico. First found in the United States, in Arizona, 1927.

Form 56. Found twice in Iowa; for the first time in the United States; isolated in 1927 by the Dominion Rust Research Laboratory.

Identity and nature of isolated physiologic forms. The reactions of 12 differential varieties to the forms isolated in 1928 are as follows: Little Club was susceptible to all forms isolated in 1928. The *vulgare* wheats, Marquis, Kanred, and Kota, were susceptible to 13, 8, and 16 of the forms,

TABLE 8.—*Prevalence and distribution of physiologic forms of Puccinia graminis tritici isolated in North America in 1928*

Distribution	Physiologic forms																			Total number of	
	1	2	11	17	18	19	21	23	29	30	32	34	36	38	39	49	50	52	56		
	Number of times isolated																			Cultures	Forms
United States																					
Alabama	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	1
Arizona	...	...	...	1	...	...	...	...	...	...	...	...	2	1	...	...	...	...	...	4	3
California	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...	2	2
Colorado	...	...	...	...	...	...	1	...	...	...	...	...	1	...	...	...	...	...	...	2	2
Georgia	...	...	...	...	...	...	...	...	...	...	...	...	1	2	...	...	...	...	...	3	2
Illinois	1	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	2	2
Indiana	...	...	...	...	...	1	...	...	...	...	2	...	3	12	2	...	...	...	...	20	5
Iowa	1	...	...	3	1	...	4	...	2	...	...	...	8	1	4	1	...	1	2	28	11
Kansas	1	...	5	...	3	2	9	...	...	...	...	...	10	2	4	3	...	...	...	39	9
Louisiana	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	1
Michigan	1	...	1	...	1	1	...	...	...	...	...	...	...	1	1	1	...	...	...	7	7
Minnesota	2	1	...	1	1	1	15	...	1	...	1	...	18	1	...	5	...	1	...	48	12
Mississippi	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	1
Missouri	...	...	...	1	...	...	1	...	...	...	1	...	5	...	...	1	...	...	...	9	5
Montana	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...	...	...	2	2
Nebraska	...	...	...	1	...	...	8	...	...	...	4	...	8	4	3	1	...	...	...	29	7
New York	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	1	...	...	...	2	2
North Dakota	1	...	...	2	...	...	3	...	...	...	1	...	8	1	...	1	...	...	...	17	7
Ohio	...	...	...	...	...	...	...	...	...	...	...	...	...	4	...	...	...	...	...	4	1
Oklahoma	...	...	...	...	1	2	2	...	...	...	1	...	3	8	...	4	...	...	...	21	7
South Dakota	...	...	...	...	1	1	9	...	...	...	...	1	16	2	1	1	...	...	...	32	8
Tennessee	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	...	...	...	...	1	1
Texas	...	...	2	...	...	1	7	...	1	...	2	...	5	20	...	4	...	...	...	42	8
Wisconsin	1	1	...	...	...	1	4	...	...	...	...	...	4	4	...	...	...	...	...	15	5
Wyoming	...	...	...	...	1	...	...	...	...	1	1	...	1	3	...	...	...	...	...	7	3
Canada																					
Manitoba	...	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	1	1
Mexico																					
Coahuila	...	...	...	...	...	...	...	1	...	...	1	...	...	7	...	4	1	...	...	14	5
Guanajuato	...	...	...	1	...	...	...	1	...	...	...	...	...	6	...	...	...	...	...	8	3
Mexico	...	...	1	...	...	...	2	...	...	...	...	...	...	6	...	1	...	...	...	10	2
Puebla	...	...	...	3	...	...	...	1	...	...	...	...	1	13	2	...	...	1	...	21	5
Queretaro	...	...	...	1	...	...	...	...	...	...	...	...	...	7	...	...	...	...	...	8	2
Number times each form was found	8	2	9	14	9	10	67	3	4	1	14	1	96	105	22	29	1	4	2	401	19

respectively. The durum varieties, Arnautka, Mindum, and Spelmar, acted as a group and were susceptible to 6 of the 19 forms. They reacted heterogeneously to 4 forms. Kubanka was susceptible to 9 forms and reacted heterogeneously to 9. Acme was susceptible to 14 forms and reacted heterogeneously to the other 5. Einkorn was susceptible to 15 forms. Of the 2 emmer varieties, Vernal was susceptible to 2 forms, and Khapli, to none.

Evidence of migration of rust from South to North: Comparison of southern and northern physiologic forms. The outstanding form in the wheat fields of Mexico and Texas in 1928 was form 38. Sixty-one identifi-

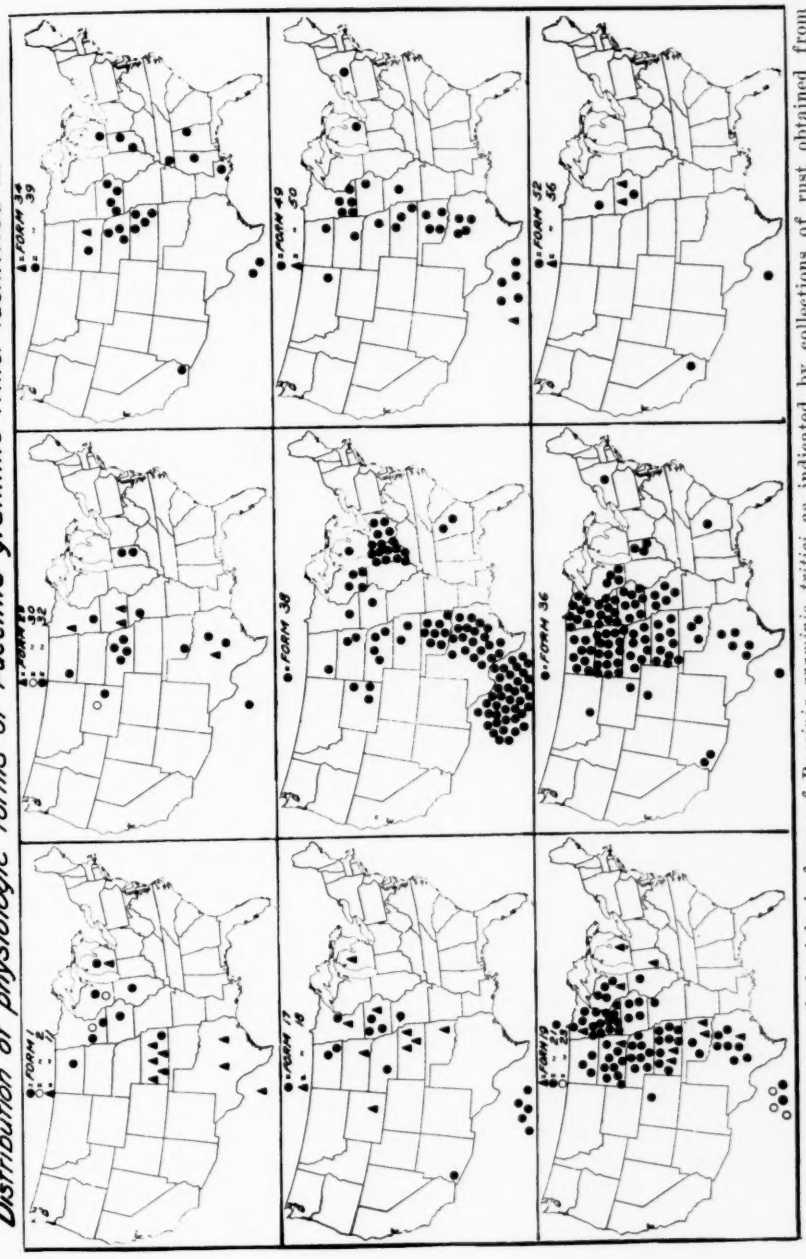

FIG. 3. Distribution of physiologic forms of *Puccinia graminis tritici* as indicated by collections of rust obtained from different States in the United States and from Canada and Mexico and identified in 1928.

cations were made from material obtained in Mexico. Form 38 was identified 39 times. In Texas this form was found in 20 of a total of 42 collections. Although not collected so frequently in the central and northern United States as forms 21 and 36, form 38 seemed to be quite generally distributed. It is the opinion of the writer and also of others who kept in close touch with the 1928 survey that the majority of the collections of form 38 made in the United States this year may have been due to a migration of this form from Mexico into Texas and then northward.

Early in March 3 collections of stem rust of wheat were made at different locations in Texas. These collections were made by Wallace Butler and were the first rust found by Mr. Butler that spring. These primary infections had produced only a few, very small pustules when collected. Upon identification each collection proved to be form 38. According to Mr. Butler,[10] stem rust of wheat developed very slowly in Texas after the first infections were found. Later, when infections became more general, a larger number of collections were received from various points in Texas. The majority of these collections also were form 38.

In May, numerous collections of stem rust of wheat were made by H. H. Thornberry while studying rust conditions in Mexico. At this time most of the Mexican wheat had ripened and was cut, but many late fields were found. In several fields, and especially near irrigation ditches, the rust was in epiphytotic form, the infection being as high as 100 per cent. As stated previously, most of these Mexican collections were identified as form 38.

The reaction of Marquis seedlings to most of the Mexican collections of form 38 was somewhat at variance with their reaction to the original collection of form 38. This atypical reaction of Marquis was studied carefully, for it was clear that should such prove to be constant it would be of value in determining whether this form migrated to the United States.

Form 38 is described as producing a light type of infection on Marquis (5). The infection produced may sometimes range from 1= to 3–, but the mean infection and the one most regularly encountered is 2=. Type 2 is characterized by the production of green islands with a small pustule in the center, and the island is surrounded by a rather sharp necrotic halo. The type 1= infection results when the pustule is extremely small and the green island is not evident. Occasionally, the pustules are considerably larger and may almost completely cover the green island. The increase in size of the rust pustule usually is associated with a chlorotic appearance of the island tissues. Although this complete range of infection may sometimes be found when Marquis is infected with form 38, the usual and rather constant reaction of Marquis to various collections of this form is the production of an ordinary type-2 pustule.

[10] Annual report, Division of Cereal Crops and Diseases—unpublished.

In the Mexican collections of form 38 it was observed that the reaction of Marquis, in many cases, was not typical. Often the infection ranged from 1 ± to 4, which was somewhat like the type-x or heterogeneous infection produced by certain forms on the durum varieties. Particular attention was given to this reaction of Marquis during the season. The collections behaving this way were identified as form 38, although it was recognized that there might be a possibility of this being a new form. Subsequent inoculations with some of the Mexican collections proved that this behavior was due at least in some instances, to a mixture of 2 forms, the typical form 38 being 1 of them and form 49 the other. Form 49 was collected 5 times in Mexico independently of form 38.

Whether all of the above collections were in reality a mixture of form 38 and form 49 or even if they were some hitherto undescribed form, apparently this form invaded the United States and Canada from Mexico. Certain collections of form 38, which gave no such variation as the Mexican form 38, were found in the United States, but a sufficient number of collections were typical of the Mexican form 38 to constitute evidence of a northward migration of stem rust of wheat in 1928. Other forms isolated from Mexican collections were found in hardly sufficient number to be used as evidence for or against migration. Forms 23 and 50 were found in Mexico 3 times and once, respectively, but were not collected in the United States. It is possible that both of these forms came into the United States from Mexico if there was a migration of rust. The fact that Marquis, Kanred, and the commonly grown durum varieties are resistant to both forms may have made it difficult for these forms to become established. In the absence of susceptible varieties one would not expect these forms to increase very rapidly, even though they may have reached the fields of the northern United States.

Only 3 other forms were found in Texas as many as 4 or more times each. Forms 21, 36, and 49 were found there 7, 5, and 4 times, respectively. In the other parts of the United States these 3 forms ranked third, fourth, and fifth in prevalence.

Again, in 1928 certain forms were present in the northern wheat fields of the United States, which apparently were extremely scarce or entirely absent from southern fields. Only 1 of these was collected a sufficient number of times to be of significance in this study. Form 39 was found twice in Mexico but was not collected in Texas or Oklahoma. It was collected 20 times in the United States and seemed to be most prevalent in Kansas, Nebraska, and Iowa.

In general, the evidence accumulated in the 1928 survey suggests that some rust may have migrated from Mexico and Texas to the northern United States and Canada. The distribution of physiologic form 38 supports this

suggestion. Possibly forms 21 and 36 migrated to some extent from Mexico or Texas, as they were extremely common in the northern wheat fields. No doubt these 2 forms also spread in the North from barberries, as they were the most common forms in the barberry area in 1927.

Comparison of United States Survey with Canadian Survey. Complete results of the survey conducted at the Dominion Rust Research Laboratory at Winnipeg in 1928 are not available. The writer was furnished with an incomplete report through the kindness of Margaret Newton and Thorvaldur Johnson of that laboratory. These data show that over 400 collections made in 1928 had been identified up to January, 1929. Forms 21 and 36 were extremely common, as they comprised almost ¾ of the identifications. Form 38 ranked third in prevalence with form 15 a close fourth. Forms 15 and 52 were fairly common, especially in the Province of Manitoba. That these last 2 forms did not spread into Canada from the wheat fields of the United States, especially those of the Mississippi Valley, is quite evident. Form 15 was not collected in the United States and form 52 was found only twice.

Whether forms 21 and 36 migrated from Texas northward is a matter of conjecture. These forms did not seem to be very prevalent in Mexico. They were, however, more prevalent in Texas, and extremely common in the northern United States. Form 38 was found rather commonly in Canada and the Canadian investigators also observed the atypical reaction of several of these collections. The same variation of the reaction of Marquis was observed by them as was noticed by the writer and others at the Minnesota laboratory.

Form 39 apparently was not present in Canada. The records show that this form has never been isolated at the Canadian laboratory. It was relatively common in the United States in 1928. If wheat stem rust migrated from the northern United States into Canada this year, the absence of form 39 in Canada may have been due to the fact that it was not present in Montana, North Dakota, Minnesota, or Wisconsin, the 4 border States of the spring-wheat region. The absence of form 39 from Canada can hardly be explained by the fact that Marquis is resistant to this form, since this would hold true for form 38 as well.

PHYSIOLOGIC FORMS EXPECTED FROM BARBERRIES IN 1928

Since form 21 and form 18 or 36 were the prevailing forms in 1927, it would be expected that these forms would develop on barberries in 1928 in greater proportion than other forms found in the fields in 1927. In the barberry area forms 21 and 36 were the most common forms in 1928. From the 1927 survey one might also expect forms 39 and 49 to originate to some extent on barberries in 1928. Form 39 was collected 7 times in Ohio in

1927 but was not collected there in 1928. Seven collections were made from Kansas in 1927 and 4 in 1928. It was not found in Iowa in 1927 but was collected there 4 times in 1928. This form was collected 33 times in 1927 and 22 times in 1928. Form 49 was collected 9 times in 1927 and, with the exception of 1 collection from Oklahoma, it was not found south of Kansas and Missouri. In 1928 this form was collected 29 times, but 13 of these collections were made in Mexico, Texas, and Oklahoma, and it is probable that, at least, these southern collections of form 49 in 1928 did not originate from aeciospores from barberry bushes. Forms 32, 17, 11, and 1 were found at various places in the barberry area in 1927, and, although they were not collected so often as forms 21, 36, 39, and 49, they were common enough in the uredinial stage to suggest that they would reappear in 1928 through the agency of the barberry. Form 38 was particularly prevalent in Ohio in 1927 and should have been one of the forms spreading from barberries in that region in 1928, if conditions were favorable for its survival. This form was collected in Ohio 4 times in 1928. Whether or not it developed there on barberries was not determined.

Forms 32, 17, 11, and 1 were found in about the same proportion in 1928 as in 1927.

In the spring of 1928 an attempt was made to identify physiologic forms that occurred naturally on barberries. Over 200 collections of aecial material were sent in by various State leaders of barberry eradication and barberry scouts. This attempt was not very successful, as it was extremely difficult to obtain fresh, viable material. When a collection was received, the infected barberry leaves were placed on moist filter-paper in Petri dishes and left until the discharge of aeciospores began. Seedling wheat, oats, barley, and rye were then inoculated. No other grass hosts were available for inoculation. Sixty-two rust cultures were obtained from a total of 213 aecial collections, from which inoculations were made. Thirty-eight of these successful inoculations proved to be *Puccinia graminis secalis;* 2 were *P. graminis avenae;* and 17 cultures of *P. graminis tritici* were obtained. Table 9 gives the results of the identifications of the cultures of *P. graminis tritici* obtained from rusted barberries. Also 2 uredinial collections made near infected barberries in Indiana and 1 made in Wisconsin are included in this table. The fact that a large majority of the aecial collections gave cultures of *P. graminis secalis* does not necessarily mean that this strain of rust was most prevalent on barberries. It is entirely possible that the aeciospores of *P. graminis secalis* are more resistant to drying and to aging than those of other varieties of stem rust.

The number of identifications of physiologic forms of *Puccinia graminis tritici* from aecial material was too small to be of much significance. It will be noted, however, that all the forms that occurred in the uredinial stage

TABLE 9.—*Physiologic forms of Puccinia graminis tritici identified in 1928 from aecial collections and from uredinial infections directly traceable to infected barberries*

State	Host	Number of		Physiologic forms							
		Collections	Cultures	1	11	17	32	36	38	39	49
Iowa	Barberry	1	1	1	...	...	...	...	...	...	...
Kansas	"	1	1	1	...	...	...	...	...	...	...
Michigan	"	4	5	1	1	...	...	...	1	1	1
Minnesota	"	3	4	2	...	...	1	1	...	...	...
Missouri	"	3	4	...	...	1	1	2	...	...	...
New York	"	1	2	...	...	...	...	1	...	...	1
Indiana	Wheat near barberries	2	3	...	...	...	...	1	2	...	...
Wisconsin	*Hordeum jubatum* near barberries	1	1	...	...	...	...	...	1	...	...
Total		16	21	5	1	1	2	5	4	1	2

with any regularity in the barberry area, in 1927, with the exception of form 21, were identified from barberry collections made in 1928.

It is of interest to note that 2 of 3 collections from rusted wheat near barberry bushes in Indiana proved to be form 38 and that this form was found in 10 other collections out of a total of 20 from Indiana.

Incomplete records from the Dominion Rust Research Laboratory show that most of the forms common there in 1927 were collected in approximately the same proportion in 1928. Some forms found in Canada in 1927 had not been identified there in 1928 when these data were obtained by the writer. Other forms had been collected in 1928 that were not collected in Canada in 1927, and this would suggest some other source than local barberries.

It is, of course, unsafe to forecast the occurrence of different physiologic forms in a particular year because of the fact that they were abundant the previous year. The writer fully appreciates that there may be numerous factors influencing the survival of teliospores from one season to the next. There may be differences in the individual physiologic forms in this respect. Before it will be safe to make such a forecast a study of individual form differences and of the origin of physiologic forms must be completed. A systematic survey of the forms developing on barberries is needed each year and, until this is done, much of the evidence accumulated on the question of the source of inoculum must necessarily remain circumstantial.

DAMAGE FROM STEM RUST OF WHEAT IN 1926, 1927, AND 1928

The estimated losses, in bushels of wheat, from stem rust in 1926, 1927, and 1928, are shown in figure 4 These estimates were obtained from the Conference for the Prevention of Grain Rust.[11] These data include only the losses from wheat stem rust in the barberry-eradication area. In all 3 years most of the rust damage was in the hard-red-spring and durum-wheat States. In only 1 of these 3 years, namely, 1927, did stem rust of wheat

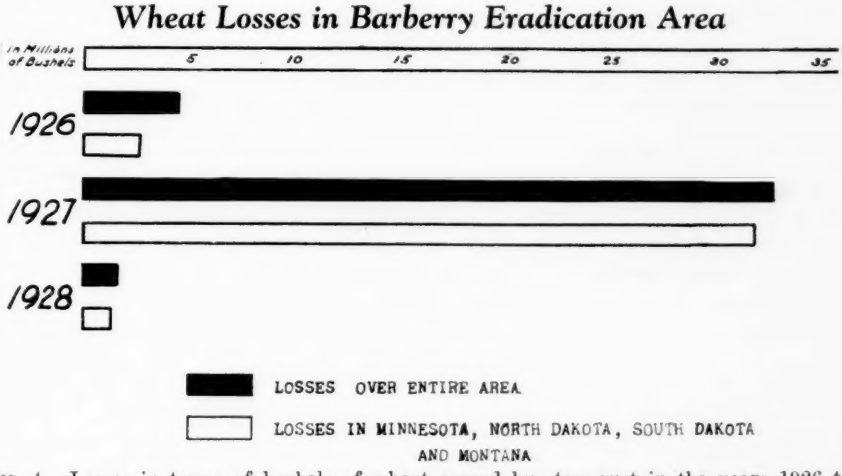

FIG. 4. Losses in terms of bushels of wheat caused by stem rust in the years 1926 to 1928, inclusive.

occur in epiphytotic form. The loss in 1926 was almost entirely due to local epiphytotics. In 1927 there was a rather general epiphytotic of stem rust and a consequent loss of over 30 million bushels. In 1928 the entire loss from stem rust was almost negligible.

DISCUSSION AND CONCLUSIONS

The evidence accumulated from the distribution and prevalence of physiologic forms of wheat stem rust in 1926 suggests that in this particular year there was no appreciable migration of inoculum from the South to the North. This would mean that most of the rust in northern fields in 1926 came from barberries in that region.

In 1927 the physiologic forms that were predominant in Texas were likewise predominant in the North, being generally distributed and extremely common. Two forms, namely, 18 and 21, appeared to be largely responsible for the epiphytotic of 1927. These 2 forms were very closely asso-

[11] The writer wishes to express his thanks to Mr. Donald G. Fletcher, Executive Secretary of the Conference for the Prevention of Grain Rust, for these data.

ciated in their distribution and in a great many instances were found together, either on the same rusted wheat or grass plant or in the same or adjoining fields. Collectively, they were able to attack all of the commonly grown wheat varieties.

In 1928 form 38 stood out as the most prevalent southern form. It was present in epiphytotic proportions in certain wheat fields in Mexico and was identified 39 times out of 61 Mexican isolations. As has been described previously in this paper, some of the Mexican collections of form 38 were not typical of the form 38 previously encountered in wheat-stem-rust surveys. The Mexican collections often produced an infection on Marquis wheat resembling a type-x or heterogeneous infection, later proving in several instances to have been due to a mixture of forms. Marquis is resistant to the normal form 38, developing a type-2 infection. Later in the season this atypical form 38 was found in the Northern States, but it was not so common as certain other forms. Collections of typical form 38 also were found in the Northern States, and in 3 instances it was identified from infected plants growing near infected barberries. In 1927 form 38 was rather common in Ohio and other eastern States and one would expect to find it the next year on barberries.

Form 38 is not considered a virulent form of stem rust in the hard-red-spring and durum-wheat regions, since Marquis is resistant and the durum varieties react heterogeneously to it. It could not produce an epiphytotic in the hard-red-spring and durum regions. Although the Mexican collections of this form occasionally produced what appeared to be a virulent type of infection on seedling leaves of Marquis wheat, the prevailing infection was of the resistant type. At the time this paper was prepared no study had been made of the reaction of more mature plants in Marquis to this Mexican form 38.

It is now generally accepted that the wind is an important agent in the dissemination of rust spores. A discussion of the wind and its rôle in the spread of rust spores is not included in this paper, since it has been discussed in considerable detail recently by Lambert (4).

It is the writer's belief that in 1928, at least, a limited amount of inoculum was blown into the wheat fields of the northern United States from southern fields and that the inoculum originally came from Mexico. Whether the inoculum came with the winds directly from Mexico or Texas or reached the northern fields after a gradual migration the writer is not able to say. It is believed, however, that form 38 would have been collected more times than it was had there been more susceptible varieties grown in the spring-wheat area and had an effort been made to identify the forms causing weak infections on Marquis wheat in the spring-wheat region. Naturally, the majority of collections were on Marquis or some of the durum varieties, and the average field man in making rust collections selects heavily

rusted material. These factors, no doubt, lessen the chances of the collection of forms to which the common varieties of wheat are resistant.

The statistics on the loss from stem rust of wheat show that in 1927 a rather severe epiphytotic occurred. This supports the contention of the writer that with the eradication of barberries severe epiphytotics are more apt to result when conditions are favorable for the migration of inoculum of virulent forms from the South to the North, for in this year only, of the 3 years under discussion, was there circumstantial evidence of a migration of virulent physiologic forms of stem rust. Future research on the epiphytology of stem rust may strengthen or disprove this theory but, for the present, the evidence so far accumulated corroborates the contention that in certain years the southern source of inoculum may be the most important contributing factor in the production of stem-rust epiphytotics in the northern United States and Canada.

SUMMARY

1. Extensive annual surveys of the prevalence and distribution of physiologic forms of *Puccinia graminis tritici* were made in 1926, 1927, and 1928 with the primary object of studying the migration of stem rust of wheat from South to North.

2. Evidence accumulated in 1926 suggests that there was no appreciable South-to-North migration of wheat stem rust in 1926, since the predominant forms in Texas were not found in the northern Mississippi Valley.

3. The loss from stem rust in 1926 was slight and only localized epiphytotics occurred.

4. In 1927, forms 18 and 21 were exceedingly common in Texas and were generally distributed over the fields of the northern Mississippi Valley. The evidence suggests a rather general northward migration of stem rust of wheat in 1927.

5. A serious epiphytotic of stem rust of wheat occurred in the spring-wheat region in 1927. The loss was over 30 million bushels.

6. In 1928, form 38 was outstandingly predominant in Mexico. In Texas it also was by far the most common form, and throughout the United States was widely distributed. The evidence was rather striking that this particular form could have originated in Mexico in 1928. It is suggested that the failure of this form to become sufficiently established to produce an epiphytotic was due to the fact that its host range is somewhat limited, as Marquis is resistant and the commonly grown durum varieties react heterogeneously to it.

7. The loss from stem rust of wheat in 1928 was insignificant. The total loss in the 13 States of the barberry-eradication area amounted to only 1½ million bushels compared with nearly 35,000,000 bushels in 1927.

8. The value of physiologic-form surveys in the study of the epiphytology of stem rust of wheat is brought out and it is further stressed that,

along with surveys of this kind, which include principally field collections of uredinial material, similar surveys that include aecial infections are essential. Surveys of the past several years suggest rather definitely that certain physiologic forms exist in the northern United States and Canada probably exclusively through the agency of the barberry.

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THE RELATION OF PENTATHIONIC ACID AND ITS COMPONENT CONSTITUENTS TO THE TOXICITY OF SULPHUR FUNGICIDES[1]

O. NEAL LIMING[2]

The toxicity of sulphur to fungi has been attributed to a number of factors. Only four of these, volatilized sulphur, sulphur dioxide, hydrogen sulphide, and pentathionic acid, are now usually considered.

This report includes the results of experiments on pentathionic acid with reference to its chemical and physical properties and its relations to the effectiveness of sulphur fungicides. Vaporized and particulate sulphur, sulphur dioxide, and hydrogen sulphide are considered component constituents in the formation of pentathionic acid. The occurrence of these constituents in sulfur fungicides, before their application to host plants and to fungi and when in contact with them, was determined, and their toxicity to various fungi was tested. The conditions under which pentathionate ions are toxic to spores of *Sclerotinia cinerea* (Bon.) Schröt. also were studied.

VAPORIZED SULPHUR

In recent years renewed interest has been taken in the study of the volatilization of sulphur and its fungicidal significance. In 1920, Barker and associates (2) supported the generally accepted theory that the fungicidal action of sulphur is due to sulphur vapor. Barker (1) continued the work and reported in 1927 that the toxic agent is not gaseous but is particulate in nature. Goodwin and Martin (4) found that sulphur is given off as a gas from the parent mass; but is not toxic to fungi in that state (5).

Nearly every investigator has been interested in the claim that sulphur exerts a toxic influence at a distance. Sulphur vapor probably plays an important part in this phenomenon, in that any condensation product would likely be evenly distributed and finely particulate in nature. The result of such processes would be a coating of very fine sulphur over the plant to which sulphur has been applied. Therefore, the conditions under which sulphur vapor is produced and the toxicity of it and related products seem important to this subject.

[1] Abridgment of thesis submitted as partial requirement for degree of Doctor of Philosophy in Plant Pathology in the Graduate School of the Ohio State University.

[2] Grateful acknolwedgments are made to Dr. H. C. Young, at whose suggestion these studies were undertaken at the Department of Botany and Plant Pathology, Ohio Agricultural Experiment Station, and to Dr. W. G. Stover for his helpful suggestions and criticisms throughout the preparation of the manuscript.

The physical and chemical nature of volatilized sulphur. The apparatus illustrated in fig. 1 was used in the following experiment. Five grams of

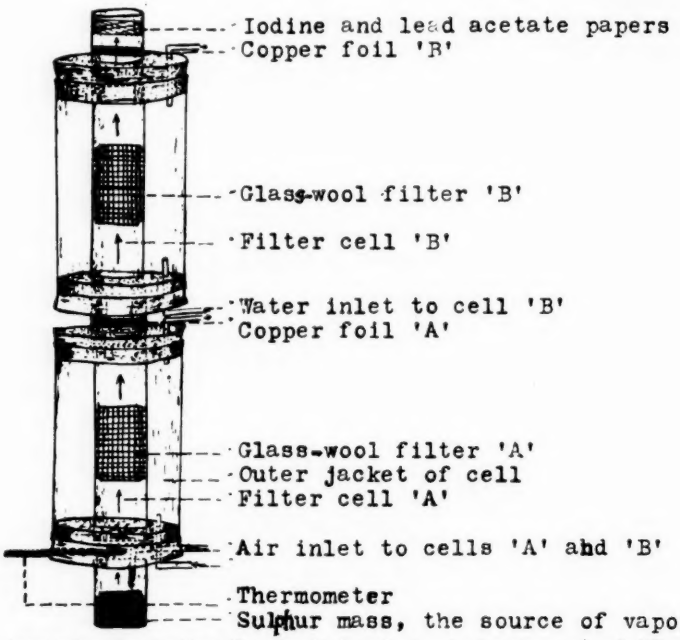

FIG. 1. Apparatus for testing the physical and chemical nature of volatilized sulphur.

sulphur were placed in the lower part of the central cell. Air washed in sulphuric acid was passed in through the air tube. A wad of glass wool was placed in the central chamber of each of the filter cells. The temperature of each filter cell was regulated by the circulation of either hot or cold water through the outer jacket. A bright copper foil was placed above the filter in each cell so that if any sulphur product passed the filter it could be detected by the formation of a tarnish on the foil. Iodine and lead acetate papers were placed at the upper end of the cells in order that the presence of sulphur dioxide and hydrogen sulphide could be detected. The conditions and results of the experiment are given in table 1.

At the lower temperatures only volatilized sulphur was formed and it passed through the hot filters. It thus appears that the volatilization product, at ordinary temperatures, is pure sulphur, of gaseous rather than particulate nature. The results of these tests agree with those of Goodwin and Martin (4).

The effect of temperature on the vaporization of sulphur. The rate of formation of tarnish on copper was used as an indication of the amount of vapor given off from a sulphur mass. The results given in table 1 show

TABLE 1.—*The physical and chemical nature of volatilized sulphur at temperatures ranging from 90 to 440° C.*

Temperature at which vapor was formed	'A' filter cell	Tarnish on copper foil 'A'	'B' filter cell	Tarnish on copper foil 'B'	Test for SO_2 and H_2S
°C.					
90	Hot	Trace	Hot	None	Negative
90	"	"	Cold	"	"
115	"	Medium	Hot	Medium	"
115	"	"	Cold	None	"
180	"	Heavy	Hot	Heavy	"
180	"	"	Cold	Trace	Positive
440	Cold	"	Cold	Heavy	"

that at temperatures of 115° and 90° C. any tarnish formed is due entirely to sulphur vapor. Preliminary tests showed that a copper foil when placed about 4.5 cm. from a mass of sulphur at 93° C. would form a gray mottle in 1 minute. For convenience in tabulating the results, all of the following tests were made at 4.5 cm.

A simple cell (Fig. 2) was made by sealing a small glass shell in the

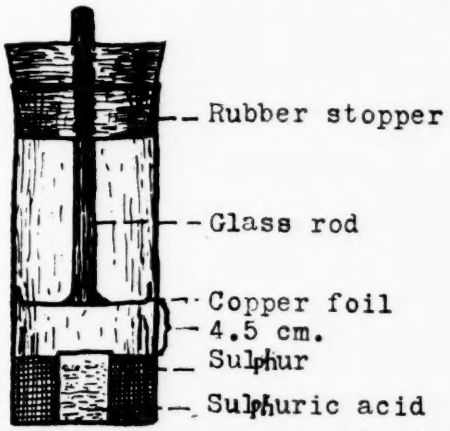

FIG. 2. Cell used to determine the rate of vaporization of sulphur and the toxicity of sulphur vapor to fungus spores.

bottom of a large tube. Copper foil was mounted on a glass rod that passed through a rubber stopper the size of the large tube. The sulphur was placed in the bottom of the cell. The small shell was filled with sulphuric acid to eliminate the effect of moisture on the tarnishing of the copper. At the start of the tests the cells, mounted copper foils, sulphuric acid, and ground sulphur were brought to the temperature desired and then quickly assembled. Each test was made in triplicate.

TABLE 2.—*The effect of temperature on the rate of vaporization of sulphur*

Temperature	Number of hours for tarnish to form			Relative rates
	Cell No. 1	Cell No. 2	Cell No. 3	
°C.				
24	310.0	326.0	342.0	1.0
26	179.0	171.0	163.0	1.9
28	98.0	95.0	95.0	3.4
30	64.0	62.5	64.0	5.1
32	47.5	47.0	47.0	6.9
35	32.0	32.0	32.0	10.2
40	15.9	17.6	16.9	19.4
50	3.9	4.2	4.2	79.0
	Number of minutes for tarnish to form			
60	61.0	60.0	62.0	317.0
70	15.0	15.0	15.5	1,304.0
80	3.8	4.0	3.8	4,910.0
93	1.0	1.0	1.0	19,560.0

The results of the tests are given in table 2. The relative rates of vaporization of sulphur at different temperatures are given in the column to the right. In the range of temperatures (24 to 35° C.) that commonly occurs during the summer the rate of vaporization varies from 1 to 10. At the higher temperatures the rate increases enormously, an increase of 70° C. (from 23 to 93°.), increasing the rate approximately 20,000 times.

The condensation and crystallization of sulphur vapor. It was observed that when sulphur was vaporized at 93° C. the vapor condensed in a very fine solid state and rapidly changed to rhombic crystals; but, when volatilized at a higher temperature (200° C.), the droplets were large and did not crystallize within 24 hours.

Sulphur, vaporized at 93° C., was condensed on a slide until the slide was milky white. Microscopic observations showed that the slide was covered with minute noncrystalline bodies. The first photomicrograph in figure 3 was taken immediately after the condensation of the vapor. In it is shown the even coverage that is often alluded to in the application of sulphur to plants by the vaporization method. The next picture of the same field was taken 30 minutes later, the third one an hour later, and the fourth, after 5 hours. Some of the drops, because of their smaller surface curvature and lower vapor pressure, apparently absorbed the vapor from the smaller drops. The larger drops then solidified and crystallized into rhombic sulphur.

The effect of light, moisture, and physical condition on the vaporization of sulphur. In these experiments the loss of sulphur from a sulphured

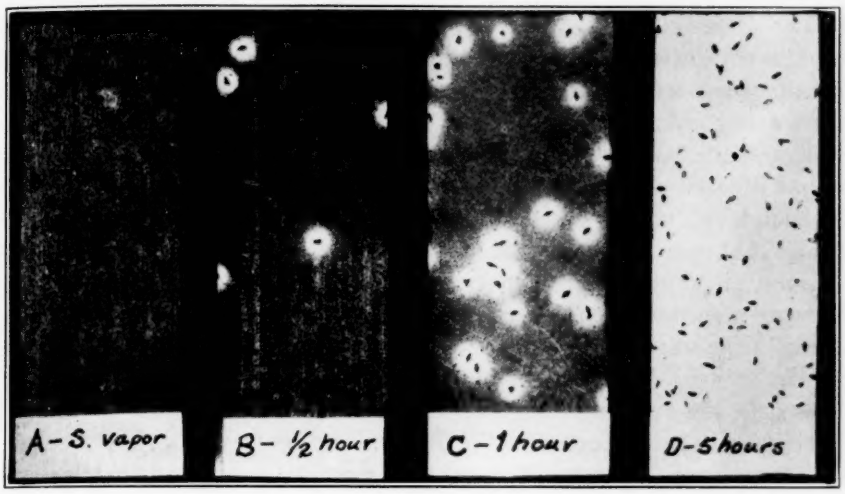

FIG. 3. The crystallization of condensed sulphur vapor on a glass slide.

glass surface by vaporization was determined by direct weighing. Ten watch crystals were arranged one above the other and held apart by small glass rings. Thus a glass tower with a large surface area (750 sq. cm.) was formed. These towers were hung in an atmosphere charged with the sulphur to be tested until each was covered with about 0.2 gm. of sulphur. They were then exposed to the various conditions mentioned above and weighed at different intervals to determine the amount of sulphur lost by vaporization.

The general results of the tests and the conclusions are all that are considered necessary to give here. The amount of sulphur lost in a dry atmosphere was not materially different from the amount lost in a humid atmosphere. The tests on the effect of light on the vaporization of sulphur were not entirely satisfactory because of the inability to control the temperature of the sulphur exposed to sunlight. It is believed, however, that light has but little, if any, effect on the rate of vaporization of sulphur. Tucker (8) and Goodwin and Martin (4) likewise concluded that these conditions have no significant effect on the rate of vaporization of sulphur.

To test the effect of the physical condition of sulphur on vaporization, flowers of sulphur, ground sulphur, and vaporized sulphur were used. The initial rate of vaporization of the vaporized sulphur at 50° C. was much greater than that of the other two types. It appears that the more finely divided crystalline form (condensed sulphur vapor) vaporizes more readily than ground sulphur and flowers of sulphur, both of which contain small amounts of amorphous sulphur.

The toxicity of sulphur vapor to fungus spores. As stated earlier, sulphur may exert a toxic influence at a distance. The assumptions are that the vapor acts directly on the fungus or that the condensation products of the vapor are toxic.

To test the toxicity of the vapor given off from a sulphur mass, cells like the one illustrated in figure 2 were used. The copper foil was removed and the round end of the glass rod was left in its place. Preliminary tests showed that spores of *Sclerotinia cinerea* and *Cladosporium fulvum* (Cke.) were not killed at a high temperature (55° C.) so readily in a dry atmosphere as when moisture was present. For this reason sulphuric acid was placed in the small shell. It was necessary to vary the temperature in the experiment to change the amount of vapor formed. The cells were first exposed in the oven until they reached a constant temperature. The end of the glass rod was touched to the dry fruiting surface of a fungus colony, and the cell was then assembled as in the previous experiment. They were then exposed to various temperatures for different lengths of time. Checks without sulphur were run at the same temperatures and under the same condition in each of the tests. After exposure to the sulphur vapor, the spores were transferred to a drop of water for germination. At the end of 16 hours the percentage of germination was determined by direct counting of the spores.

The conditions of the experiment and the results are recorded in table 3. The percentage of germination was quite variable; apparently, some spores being more sensitive to a high temperature than others in the same culture. The results are, however, sufficiently constant to indicate that sulphur vapor

TABLE 3.—*The toxicity of sulphur vapor to fungus spores*

Temperature at which vapor was formed	Minutes of exposure	Percentage of germination	
		Treated spores	Untreated spores
°C.	*Sclerotinia cinerea*		
30	20	90.3	92.3
40	10	69.0	78.1
55	5	50.5	46.7
65	2	65.7	62.9
	Cladosporium fulvum		
45	2	42.0	46.0

was not toxic to the fungus spores. Although the amount of sulphur vapor was increased at 65° C. to about 100 times the amount formed at 30° C. no toxicity due to the sulphur vapor was apparent. Goodwin and Martin (5) found sulphur vapor was not toxic to mildew fungi.

The toxicity of the condensation products of sulphur vapor. Even though sulphur vapor is not toxic to fungus spores, it is still possible that the condensed vapor or recrystallized sulphur is toxic or that it is chemically reactive and readily forms the toxic factor. To test this point sulphur vapor was condensed on glass slides until they were milky white. Drops of water containing spores were placed on the slides and they were then inclosed in a Petri dish and left for a 16-hour germination period. In other tests the slides were left exposed to laboratory conditions for 24 hours before the water and spores were added.

The results are given in table 4. The condensed sulphur vapor or recrystallized sulphur did not prevent germination of spores of *Sclerotinia cinerea.* However, through the formation of the toxic factor, the recrystallized sulphur became toxic on standing and reduced the percentage of germination below that of the check in all tests.

TABLE 4.—*The toxicity of condensed sulphur vapor, at various lengths of time after vaporization, to spores of sclerotinia cinerea*

Temperature at which vapor was formed	Hours after vaporization	Percentage of germination
°C		
80	0	89.4
80	24	27.4
110	0	91.0
110	24	12.2
200	0	75.7
200	24	0.9
Check on untreated slide		88.8

SULPHUR DIOXIDE

The theory that sulphur dioxide is the toxic factor of sulphur fungicides has not been supported by recent investigations. Williams and Young (10) found only minute traces of sulphur dioxide associated with ground sulphur. Liming and Young (6) showed that a much greater concentration than is found in ground sulphur is necessary for any significant toxicity to spores of *Sclerotinia cinerea.*

The oxidation of sulphur by the oxygen of the air is probably sufficient to account for the small amount of sulphur dioxide present. It is recognized that there may be formed on the sulphur particle a layer of S = O that gradually changes to sulphur dioxide by further oxidation. Sulphur dioxide appears to be a transitory product and, as such, the source of the acids of sulphur. Field experiments with sulphur dusts treated with manganese dioxide, an oxidizing agent, indicate that sulphur dioxide and thiosulphates are formed and then reduced to the toxic factor.

The production of sulphur dioxide in sulphur. Tests were made to determine what natural factors affect the accumulation of sulphur dioxide in sulphur. For each test, 25 gm. of sulphur were placed in a 50 cc. flask, treated in various ways, and then set away for 5 months under different conditions. At the end of the tests, 25 cc. of water were added to each of the flasks and the contents mixed thoroughly. Each suspension was divided into two equal parts, one was titrated directly with iodine, and the other was treated with formaldehyde and then titrated. The difference in the amount of iodine used in the two fractions indicated the amount of sulphur dioxide in the suspension.

TABLE 5.—*The effect of time, light, moisture, high temperature, and oxygen on the accumulation of sulphur dioxide in sulphur*

Light wet 23° C.	Light wet oxygen 23° C.	Light dry 23° C.	Light dry oxygen 23° C.	Oxygen dark wet 45° C.	Oxygen dark wet 23° C.	Oxygen dark dry 45° C.	Oxygen dark dry 23° C.	Dark wet 45° C.	Dark dry 45° C.
x[a]	x	x	x	x	x	x	x	x	x
x	xxx	xx	xxx	xx	x	x	xx	x	x

[a] x indicates trace, xx light, and xxx a medium amount of SO_2.

The symbols used in the table indicate the relative amount of sulphur dioxide present in the solutions. At the beginning of the experiment a trace of sulphur dioxide was found in checks to all of the tests. Sulphur treated with oxygen did not give consistent results unless it was exposed to light. Oxygen and light were the only factors that gave consistent evidence of increasing the amount of sulphur dioxide in sulphur.

The toxicity of sulphur dioxide to fungus spores. Tests were made to determine the sensitivity of spores of *Sclerotinia cinerea* to sulphur dioxide. A quantity of the gas, which had been washed in sulphuric acid, was measured into an Erlenmeyer flask. The dry spores were held on a dry glass rod, as in the toxicity tests of sulphur vapor. The flask was then closed with the stopper that held the glass rod. The spores were located as near the center of the flask as possible. The flask was continuously turned and rotated during the time of exposure. The spores were then removed and placed in distilled water for germination. At the end of the tests the gas was slowly forced into a large flask partly filled with water which took up the sulphur dioxide. The transfer was made so as to determine only the amount of gas in the atmosphere in the original flask. The amount adsorbed on the walls of the flask, and so not in the atmosphere, was not measured. The solution was next titrated with iodine.

TABLE 6.—*The toxicity of sulphur dioxide to spores of Sclerotinia cinerea*

Percentage of sulphur dioxide in atmosphere	Duration of exposure and percentage of germination								
	Seconds			Minutes					Hrs.
	10	20	30	1	2	3	5	10	5
9.7	49.8	0.0							
4.7	87.1	60.1	12.2	0.0					
2.3	95.4	90.1	74.0	51.2	0.0				
0.97		85.4	73.7	68.0	28.1	0.0			
0.50				89.7	87.6	76.4	10.2	0.0	
0.19						90.1	87.1	57.2	
0.01									39.2
Check in washed air									90.8

The results are given in table 6 as the percentage of germination of spores exposed to the gas for the time and at the concentration indicated. Germination of 39.2 per cent of the spores occurred after 5 hours' exposure to a 1–10,000 concentration. The results of the tests, which lasted for only a few seconds, are probably not accurate, since it is possible that the atmosphere was not thoroughly mixed during the test. However, the results indicate that spores of *Sclerotinia cinerea*, which are very sensitive to sulphur fungicides, are probably not at all sensitive to sulphur dioxide at concentrations in which it occurs in ground sulphur.

The toxicity of sulphurous acid to spores of Sclerotinia cinerea. Moist conditions necessary for spore germination are favorable to the formation of sulphurous acid from sulphur dioxide. Therefore, toxicity tests were made with 1.5 M solutions of sulphurous acid (saturated aqueous solutions of sulphur dioxide) and with solutions as dilute as 0.07 M. The spores were germinated in smear cultures on cover glasses inverted over van Tieghem cells, as described by Liming and Young (6), except that 10 cc. of the solution tested were placed in the bottom of the germination chamber. The solutions were buffered with a H_3PO_4–NaOH solution to eliminate the toxic effect of the hydrogen ions.

TABLE 7.—*The toxicity of sulphurous acid to spores of Sclerotinia cinerea*

Molecular concentration	Percentage of saturation	Percentage of germination
1.5	100	10.5
0.3	20	89.9
0.2	13	92.6
0.07	5	92.1
Check in buffer solution		93.1

A 0.3 M. solution of sulphurous acid, buffered to pH 5.4, was found to be the maximum concentration in which germination of spores was not inhibited. In the tests in which the spores did not germinate the toxicity can probably be attributed to the high molecular concentration of the solution. It is concluded that the sulphite ion is not toxic to spores of *Sclerotinia cinerea*.

HYDROGEN SULPHIDE

Barker (1), while working on the volatility of sulphur, noticed that hydrogen sulphide is formed from sulphur when dusted on living leaves. Marsh (7) confirmed this work and further observed that the gas is produced when sulphur is dusted on fungus colonies or mixed with fungus spores. Wilcoxon and McCallen (9) support the English workers.

Hydrogen sulphide does not exist in ordinary ground sulphur (10). If hydrogen sulphide is formed from the hydrolysis of sulphur in the presence of moisture it apparently reacts at once with the other products of hydrolysis and the oxides to form more complex compounds. The sulphur dioxide associated with sulphur must be reduced if pentathionic acid is formed from it. Hydrogen sulphide is the most likely reducing agent, and, although not existing in ground sulphur, it may be an important transitory product.

The production of hydrogen sulphide in ground sulphur. Several tests were made to determine the effect of natural conditions on the production of hydrogen sulphide in ground sulphur. Flasks were prepared as in the first experiment with sulphur dioxide and then treated as indicated in table 8. The presence or absence of hydrogen sulphide at the end of 5 months was determined by placing a lead-acetate paper on the sulphur mass.

TABLE 8.—*The effect of time, light, moisture, and high temperature on the accumulation of hydrogen sulphide in sulphur*

Light wet 23° C.	Light dry 23° C.	Dark wet 23° C.	Dark wet 45° C.	Dark wet 110° C.	Dark dry 45° C.	Dark dry 110° C.	Dark dry 23° C.
0	0	0	0	0	0	0	0
0	0	0	0	x	0	–	0

0 indicates no hydrogen sulphide; x indicates a trace of the gas.

In all of the tests except one the lead-acetate tests were negative. Wet sulphur, kept in the dark 5 months, at 110° C., gave a positive test for hydrogen sulphide, but only a trace was formed. Conditions, such as light, moisture, and heat, to which ground sulphur is exposed in the field, apparently, have no marked effect on the formation and accumulation of the gas in ground sulphur.

The effect of higher plants on the production of hydrogen sulphide. When sulphur is dusted on living plants hydrogen sulphide is formed and may be given off as a gas. Tests were made to determine to what extent the gas is formed when sulphur is dusted on living leaves. A simple chamber was made by placing a rubber ring between the 2 parts of a Petri dish, thus holding them apart the width of the ring. To the ring was glued a piece of cheese-cloth, so that, when assembled, the chamber was divided into 2 parts by the cloth. A section was cut out of the rubber ring to make a hole for the petiole of the leaf. In the bottom of the chamber was placed a lead-acetate paper and to the top were glued 2 glass rings with cover-glasses. These glasses were used to hold drops of distilled water very near the sulphured leaf. The leaf was dusted with ground sulphur and then enclosed in the chamber as indicated in figure 4. The dusted leaf was not in contact

FIG. 4. Cell used in testing the toxicity of hydrogen sulphide, produced from sulphur on apple leaves, to spores of *Sclerotinia cinerea.*

with the glass or the lead-acetate paper and was only lightly supported by the cloth diaphragm. The test chambers were opened at the end of 24 hours. The drops of water on the cover-glasses were tested for a change in acidity; the change was never greater than that in drops held over non-dusted leaves. The degree of coloration of the lead-acetate paper was taken as an indication of the relative amount of hydrogen sulphide formed. The results given in table 9 show that during a 24-hour period never more than

a trace of hydrogen sulphide was produced from sulphur by any of the leaves, excepting those of the bean and strawberry.

TABLE 9.—*The effect of higher plants on sulphur with reference to the production of hydrogen sulphide*

Species of plant and side of leaf tested	H_2S from sulphured leaves	Species of plant and side of leaf tested	H_2S from sulphured leaves
Both sides of leaf		*Lower side of leaf*	
Cottonwood	x	Bean	x
Geranium	0	Strawberry	x
Apple	x	Red Oak	0
Tomato	0	Maple	0
Elm	0	Apple	x
Peach	x		
Cucumber	x	*Upper side of leaf*	
Strawberry	xx	Rose	x
Onion	0	Strawberry	x
Bean	xx	Bean	0
Blue grass	x	Cyclamen	x
Dahlia	x	Cucumber	0
Potato	0		

0 indicates none, x trace, and xx light.

The effect of fungi on the production of hydrogen sulphide from sulphur. Twenty-three species of fungi were tested to show the relation between sulphur and the type of fungus in the production of hydrogen sulphide. Phycomycetes, ascomycetes, basidiomycetes, and deuteromycetes, and saprophytic as well as parasitic fungi were included in the group. Fungi causing plant diseases controllable with sulphur and those not subject to such control were tested. These types of fungi were tested in order to gain some idea concerning the relation between the production of hydrogen sulphide by a fungus and the control with sulphur of the disease it causes.

In each test the fungus was transferred to an agar plate and allowed to grow until the colony almost filled the plate. The fungus colonies were then heavily dusted with acid-free sulphur. A lead-acetate paper was glued in the top of each of the Petri dishes. The tests were run for 3 days, at the end of which time the color of the lead-acetate papers was taken as an indication of the relative amount of hydrogen sulphide formed.

The results of the experiment are recorded in table 10. All of the fungi produced hydrogen sulphide from sulphur. However, there seems to be no correlation between the rate of hydrogen sulphide production and the type of fungus tested.

TABLE 10.—*The effect of fungi on sulphur with reference to the production of hydrogen sulphide*

Fungi tested	H_2S from sulphured colonies	Fungi tested	H_2S from sulphured colonies
Sclerotinia cinerea	xxx	*Alternaria* sp.	xxx
S. sclerotiorum (Lib.) Bounck.	xxx	*Fusarium lini* Bolley	xx
Cercospora beticola Sacc.	x[a]	*F. conglutinans* Wollenw.	xxx
Willow canker fungus	xxx	*Phoma pomi* Pass.	xxx
Coccomyces hiemalis Higgins	xx	*Penicillium* sp.	xxx
Rhizoctonia solani Kühn	xxxx	*Aspergillus* sp.	xxx
Cladosporium fulvum	x	*Phomopsis* sp.	x
Armillaria mellea Vahl.	xx	*Glomerella cingulata* (Stonem.) S. & v. S.	xxxx
Venturia inaequalis (Cke.) Wint.	xxx	*Colletotrichum lindemuthianum* (S. & M.) B. & C.	xx
Botrytis cinerea Pers.	xxxx		
Rhizopus nigricans Ehrenb.	xxxx		

[a] x indicates trace, xx light, xxx medium, and xxxx heavy.

In another series of tests, 12 fungi were treated with sulphur dusts carrying different amounts of pentathionic acid. In these tests young colonies of the fungus were dusted with the sulphur and the relative amount of hydrogen sulphide produced was determined as in the above tests. At the end of 3 days the increase in diameter of the colony and the intensity of coloration of the lead-acetate paper were observed in each case. The tests of *Sclerotinia cinerea* (Fig. 5) illustrate the effects of the different sulphur

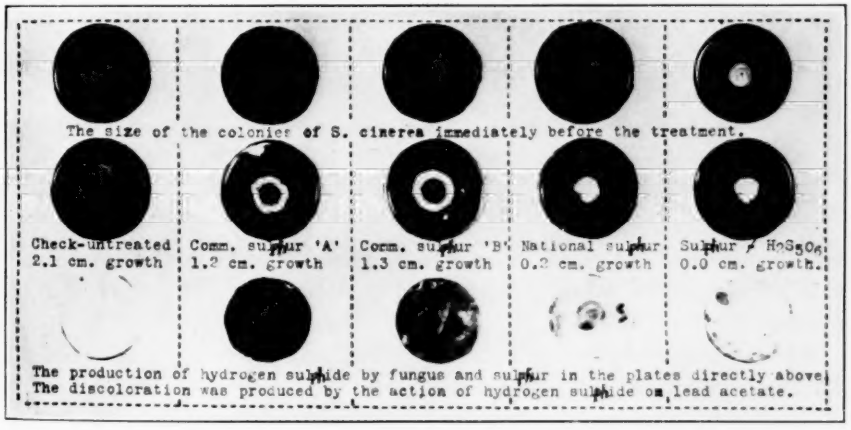
The size of the colonies of S. cinerea immediately before the treatment.
Check-untreated
2.1 cm. growth
Comm. sulphur 'A'
1.2 cm. growth
Comm. sulphur 'B'
1.3 cm. growth
National sulphur
0.2 cm. growth
Sulphur + $H_2S_5O_6$
0.0 cm. growth.
The production of hydrogen sulphide by fungus and sulphur in the plates directly above.
The discoloration was produced by the action of hydrogen sulphide on lead acetate.

FIG. 5. The relation of hydrogen sulphide and of pentathionic acid to the growth of *Sclerotinia cinerea*, determined by treating matched colonies of the fungus with sulphur dusts loaded with various amounts of pentathionic acid and 3 days later measuring the growth of the colonies and the amount of hydrogen sulphide produced.

dusts on the growth of the fungus and the amount of hydrogen sulphide produced.

The colonies dusted with sulphur carrying a large amount of pentathionic acid were killed or stunted and produced but a slight amount of hydrogen sulphide, while those treated with sulphur with only a small amount of pentathionic acid continued growth and produced a large amount of the gas. Thus, it is concluded that the production of hydrogen sulphide from sulphur cannot be correlated with the toxicity of sulphur to the fungi tested.

The toxicity of hydrogen sulphide in the gaseous condition. The results of preceding experiments indicate that hydrogen sulphide is not toxic to the fungi in the fruiting and vegetative stages. There is the possibility, however, that the gas has an adverse effect on fungus spores and affects their germination. The toxicity of hydrogen sulphide to spores of *Sclerotinia cinerea* was tested in the same manner as was sulphur dioxide.

The results of the toxicity tests are tabulated in table 11. Spores exposed to a 1–2,000 atmosphere for 5 hours showed no effects of the gas. Although

TABLE 11.—*The toxicity of hydrogen sulphide to spores of Sclerotinia cinerea*

Percentage of H_2S in atmosphere	Duration of exposure and percentage of germination									
	Seconds			Minutes				Hours		
	10	20	30	1	5	15	30	1	2	5
25.40	90.1	56.4	0.0	6.1						
10.40		96.2	89.8	58.0	0.0	7.6				
2.80				92.0	87.1	40.1	0.0			
0.97						89.0	76.8	25.9	0.0	
0.47							86.1	86.0	0.0	
0.10								92.3	89.8	
0.05										92.1
Check in distilled water after exposure to room air										91.5

the influence was not great, traces of the gas and short exposures to concentrated atmospheres actually stimulated germination of the spores of the fungus.

The toxicity of hydrogen sulphide in aqueous solution. The toxicity of aqueous solutions of hydrogen sulphide was tested in the same manner as was the sulphurous acid. The concentration of each solution is given in the molecular concentration and the percentage of saturation. In the tests the solutions were buffered with a H_3PO_4–NaOH solution to eliminate the toxic effect of the H-ions.

The results, recorded in table 12, show that relatively strong solutions (60 per cent saturation) of hydrogen sulphide buffered to pH 5.4 are not toxic to spores of *Sclerotinia cinerea.*

TABLE 12.—*The toxicity of aqueous solutions of hydrogen sulphide to spores of Sclerotinia cinerea in the presence of a buffer*

Molecular concentration	Degree of saturation	Germination
	Per cent	*Per cent*
0.140	100	0.0
0.112	80	54.4
0.084	60	93.7
0.056	40	96.0
0.028	20	98.1
Check in distilled water		95.2

PENTATHIONIC ACID

The theory that pentathionic acid is the toxic factor of sulphur fungicides was first advanced by Young (11). Young and Williams (12) showed that pentathionic acid is associated with ground sulphur and flowers of sulphur. Results given in the paper by Liming and Young (6) indicate that, of the acids of sulphur, only the polythionic acids are toxic to fungus spores. They also show that when manganese dioxide, an oxidizing agent, is added to sulphur a more effective fungicide is obtained.

The occurrence of pentathionic acid. Pentathionic acid is the principal acid in a sulphur sol formed by the reaction of hydrogen sulphide and sulphur dioxide in water. One gram of sulphur from this sol was found to carry about 0.14 millimol of pentathionic acid (3, pp. 615–625). The acid is also formed by the decomposition of a thiosulphate by an acid. This type was found to carry about 0.45 millimol of pentathionic acid per gram of sulphur (3). The acidity of pure ground sulphur is due almost entirely to sulphuric and pentathionic acids. One gram of National 300-mesh sulphur was found to carry about 0.0002 millimol of pentathionic acid. The amount of acid carried by the different types of sulphur may be varied greatly from the figures given above by varying the methods of preparation and the size of the sulphur particles.

The preparation of pentathionic acid. Solutions of pentathionic acid for toxicity tests have been obtained from the 3 sources mentioned above. Concentrated sulphurous acid was reduced with hydrogen sulphide until little but colloidal sulphur and pentathionic acid were left in the mixture. To free it from hydrogen sulphide and sulphur dioxide it was aerated for several hours until the odor of the gases had disappeared. Then the colloidal sulphur was removed by ultra-filtration. Qualitative tests showed

that only sulphur acids, principally pentathionic, with some tetrathionic and traces of sulphuric, were present. In general, 20 liters of hydrogen sulphide, when bubbled slowly into a liter of concentrated sulphurous acid, will make a 3 per cent or 0.25 N. solution of pentathionic acid.

From a solution of pentathionic acid made by the sodium thiosulphate-hydrochloric acid reaction, sodium and barium pentathionates were prepared. The salts were crystallized out in alcohol and were then taken up in water and recrystallized until, in the case of the barium salt, it was 98.6 per cent pure barium pentathionate. In 1 test, about 12 per cent of the sulphur from the thiosulphate was recovered as sodium pentathionate, and 20 per cent as the barium salt. For toxicity tests, pentathionic acid was prepared from this salt by precipitating out the barium as a sulphate.

National 300-mesh sulphur was thoroughly wet with just enough water to make a thick paste. Filtrates, which were about 0.006 N. with respect to hydrogen-ion concentration, were then taken from this paste. Quantitative tests showed that about 92 per cent of the acidity was due to sulphuric acid and 8 per cent to pentathionic acid. This filtrate was used in toxicity tests in which pure sulphuric acid of the same normality was used as a check.

The stability of pentathionic acid. There is a great difference of opinion concerning the stability of pentathionic acid. It is generally reported that strong acids and alkalies decompose the acid. Concentrated solutions of alkalies were added to concentrated solutions of the acid in the preparation of the pentathionate salts and little or no decomposition occurred. Strong alkalies when added to dilute solutions of the acid, however, destroy the pentathionate ion with the deposition of sulphur. Likewise, ammonium hydroxide destroys the acid and causes the precipitation of sulphur. Pentathionic acid, approaching the strength of concentrated sulphuric acid, has been prepared in this laboratory and seems quite stable, the only signs of decomposition being the formation of a slight amount of sulphur and sulphur dioxide. Nitric acid causes the formation of sulphur and sulphate from pentathionic acid, but this reaction is apparently due to the oxidizing properties of nitric acid. It is concluded from a consideration of these observations that pentathionic is a relatively stable acid. The barium, potassium, and sodium salts are very stable when kept at room temperatures and in a dry condition. Barium pentathionate, which has been kept in the laboratory in an open-mouth bottle for 6 months, has shown no signs of decomposition.

The volatility of pentathionic acid. The possibility that pentathionic acid is volatile was suggested by Young (11). Because of its odor and its ability to tarnish copper suspended over the acid, it appears that pentathionic acid is volatile to a certain degree. Sulphuric acid, although generally referred to as nonvolatile, will give off enough vapor to tarnish copper and to kill spores placed over a concentrated solution of the acid. Dilute

sulphuric and pentathionic acids do not show such volatility. Due to the decomposition of pentathionic acid on distillation, the degree of its volatility cannot be determined by fractional distillation.

Some indication of the volatility of the acid was obtained through reducing the volume of a solution by evaporation over calcium chloride and comparing the normality of the resulting solution with the calculated normality. If an acid is volatile the normality of the solution will not increase in proportion to the reduction of volume. After 36 hours, 2.3 per cent of a 0.25 N. solution of pentathionic acid could not be accounted for in tests in which the area of the evaporating surface was double the volume of the solution. But the possibility of any reaction that would influence the titratable acidity of the solution must be eliminated before this figure can be accepted as representing the volatility of pentathionic acid.

The toxicity of sulphur acids. The results of toxicity tests with various sulphur acids at different concentrations are presented here in table 13.

TABLE 13.—*The toxicity of acids of sulphur to spores of Sclerotinia cinerea in solutions buffered to pH 5.4. Results taken from Liming and Young (6)*

Concentration in normality	Percentage germination				
	H_2SO_4	H_2SO_3	$H_2S_2O_6$	$H_2S_3O_6$	$H_2S_5O_6$
0.1000	84.2	91.1	49.8		0.0
0.0100	86.4	93.2	90.1	12.9	0.0
0.0010	96.1	92.0	90.2	20.8	trace
0.0005	94.7	95.5	89.6	74.2	12.4
0.0001	93.7	93.6	95.4	87.2	62.6
Check	93.7	93.1	94.9	92.7	92.6

For detailed descriptions of the methods and conditions of the experiment the former paper (6) may be consulted. The trithionic acid solutions were contaminated with pentathionic acid and sulphur, except when very dilute. The pentathionic acid solutions, which were prepared by the hydrogen sulphide-sulphur dioxide method, as stated earlier, contained some tetrathionic acid.

The results show that the sulphate, sulphide, and dithionate ions are not toxic, while the polythionate ions are toxic. The relative degree of toxicity appears to be in the order; pentathionate, tetrathionate, and trithionate. A 0.001 N. solution that contains about 0.013 per cent pentathionic acid prevented germination of nearly all of the sports of *Sclerotinia cinerea*, and a 0.0005 N. solution reduced germination to 12.4 per cent.

The toxicity of pentathionic acid to fungus spores. In connection with toxicity tests of other fungicides further tests have been made with penta-

thionic acid prepared from the barium salt. In these tests the spores were suspended in a solution that contained mannite in addition to the usual buffer salts. Altogether, 9 pathogenic fungi were tested. The apparatus and the methods for the tests were essentially those used in the earlier tests. The names of the fungi and the materials tested are given in table 14.

TABLE 14.—*The toxicity of pentathionic acid to spores of pathogenic fungi, in a buffer-mannite solution*

Pathogenic fungi tested	Percentage of germination		
	Pentathionic acid		Sulphuric acid
	0.0068 N.	0.0068 N.	0.0068 N.
Verticillium alboatrum R. & Ber.	0.0	20.8	98.6
Sclerotinia cinerea	0.0	60.0	97.1
Phomopsis sp.	0.0	67.5	87.4
Graphium ulmi Schwartz	0.0	8.7	70.9
Glomerella cingulata	0.0	71.3	91.3
Botrytus cinerea	0.0	97.0	98.3
Fusarium moniliforme Sheld.	3.3	43.9	89.1
Thielavia basicola Zopf.	0.0	29.1	69.9
Phoma pomi	0.0	55.1	96.8

With but two exceptions the percentage of germination was determined at the end of 18 hours. *Verticillium alboatrum* and *Phomopsis* sp. required 48 hours for satisfactory germination.

The results of the tests show that pentathionic acid is toxic to the spores of all of the fungi tested. The degree of toxicity to the various fungi varied considerably; this, of course, being expected. The presence of mannite in the solutions made the conditions more favorable for the germination of *Sclerotinia cinerea* spores, and, consequently, about twice the usual amount (6) of pentathionic acid was required to prevent germination.

The toxic action of sulphur at a distance. Tests were made to determine the effect of 'distance' on the toxicity of sulphur and of pentathionic acid to fungus spores. In each test a spore suspension was placed 4 mm. above the material to be tested, and in a closed cell to eliminate the effect of external conditions. The duration of the experiment was 16 hours.

The results, given in table 15, show that during a 16-hour period sulphur did not exert a toxic action at a distance of 4 mm. However, a 0.25 N solution of pentathionic acid was sufficiently volatile to prevent germination of spores in a suspension held 4 mm. above the acid. It is thus possible to account for the toxicity of hydrophilic sulphur sols at a distance (11). A more dilute solution (0.05 N.) did not exert a noticeable toxic action at the distance used in the tests.

TABLE 15.—*The effect of 'distance' on the toxicity of sulphur and of pentathionic acid to spores of Sclerotinia cinerea*

Materials tested	'Distance'	Germination
	mm.	*Per cent*
Dry ground sulphur	4	87.6
Wet " "	4	84.2
0.025 N. pentathionic acid	4	0.0
0.005 N. " "	4	92.0
Check over distilled water	4	88.9

In the light of the results of this experiment and those on the production and toxicity of sulphur vapor and hydrogen sulphide, the toxicity of a sulphur at a distance cannot be attributed to any of these factors. It is believed that the movement of sulphur as a vapor and its subsequent oxidation are largely responsible for the toxic action of sulphur over a space. Hydrogen sulphide is probably an important factor in that higher oxides, which are formed from the translocated sulphur, may be reduced by it to the toxic factor.

The toxicity of sulphur filtrates. The toxicity of sulphur filtrates to spores of *Sclerotinia cinerea* was reported in the earlier paper (6). The tests were repeated, using 0.006 N. filtrates from ground sulphur and 0.008 N. filtrates from flowers of sulphur. The sulphurs from which the filtrates were taken were then treated with nitric acid and washed until the sulphur was neutral in reaction. Filtrates were then taken from these acid-free sulphurs, adjusted to pH 2.5 with sulphuric acid, and tested along with the first sulphur filtrates.

TABLE 16.—*The toxicity of sulphur filtrate to Sclerotinia cinerea spores*

Materials used in the tests	pH	Filtrates normality	Condition of sulphur when taken	Germination
				Per cent
Ground sulphur	2.5		Acid-free sulphur	90.1
" "		0.006	Acid "	11.0
Flowers of sulphur	2.5		Acid-free "	89.8
" " "		0.008	Acid "	7.6
Check in sulphuric acid		0.008		91.1

From the results recorded in table 16, it is concluded that sulphur filtrates, in which, as stated earlier, about 8 per cent of the acidity is due to pentathionic acid, are toxic; whereas filtrates from acid-free sulphur are not toxic to spores of *Sclerotinia cinerea*.

The effect of oxygen on the toxicity of sulphur. The results of previous tests (6) showing the effect of oxygen on the production of the toxic factor of sulphur are given here. For details of the methods and conditions the original descriptions may be read.

TABLE 17.—*The effect of oxygen on the production of the toxic factor of sulphur. Results taken from Liming and Young (6)*

Materials tested	Conditions	Germination
		Per cent
National 300-mesh sulphur	Oxygen absent	0.0
Acid-free sulphur	" "	46.5
Check in distilled water	" "	47.2
National 300-mesh sulphur	" present	0.0
Acid-free sulphur	" "	8.7
Check in distilled water	" "	92.7

Spores in the presence of acid-free sulphur in an oxygen-free air germinated as well as when no sulphur was present. It is therefore shown that sulphur itself is not toxic to fungus spores. The results indicate that, through the natural oxidation of sulphur in the presence of the oxygen of the air and water, the toxic factor is formed.

CONDITIONS AFFECTING THE TOXICITY OF PENTATHIONIC ACID

The conditions under which sulphur fungicides are most effective have not been studied in great detail. The results of field tests made by Liming and Young (6) indicate that alkaline sulphur dusts are not so effective as acid sulphur dusts. In other tests they show that neutral solutions of sulphur compounds are not toxic, whereas comparable solutions, when acidified, are toxic.

The effect of H-ion concentration on the toxicity of pentathionate ions. Tests to determine the effect of H-ion concentration on the sensitivity of spores of *Sclerotinia cinerea* to the toxic action of pentathionic acid were made and reported in an earlier paper (6). The results have been rearranged and are presented here to show the relative effect of the acid ions in neutral and in acid solution.

The results which are given in table 18 show that pentathionate ions do not exert a toxic effect on the spores in neutral solutions, while in acid solutions (pH 5.4) the pentathionate ions are toxic.

The stability of pentathionate ions in the presence of fungus spores. In the preceding experiment the pentathionate ions were toxic at a reaction that is below the isoelectric range (about pH 6.0) of the fungus material, and they were not toxic at a reaction above that range. It appears, there-

TABLE 18.—*The effect of H-ion concentration on the sensitivity of spores of Sclerotinia cinerea to the toxic action of pentathionate ions*

Normality of pentathionic acid solutions	Percentage germination	
	Buffered at pH 5.4	Buffered at pH 7.0
0.1000	0.0	28.2
0.0100	trace	27.8
0.0010	0.0	34.2
0.0005	7.2	29.4
0.0001	72.1	22.7
Check	91.9	31.2

fore, that either the stability of the pentathionate ions or the condition of the fungus material is the controlling factor in the toxic action. The evidence so far points strongly to the latter condition.

Tests were made over a range from pH 3.0 to pH 8.4 with a 0.001 N. solution of pentathionic acid. In each test about 1 gram of spore material was placed in 5 cc. of the acid for 6 hours. By this time the spores cultured in solutions buffered to pH 7.0 had produced short germ tubes. The spore suspensions were then filtered and the spores washed with a buffered solution of sulphuric acid to remove the free pentathionic acid from the spore mass. The filtrates and the spore masses were tested for the presence of sulphide, sulphite, and pentathionate ions.

The results of the chemical tests for the various ions are recorded in table 19. In all solutions, in which hydrogen sulphide was found, the

TABLE 19.—*The effect of fungus spores on the stability of pentathionate ions. 0 indicates none, x a trace, xx light, xxx medium, and xxxx heavy*

Normal conc.	pH	Compounds present in filtrates			Compounds present in spore masses		
		H_2S	SO_2	S_5O_6	H_2S	SO_2	S_5O_6
0.001	3.4	0	0	xxxx	0	0	xxxx
0.001	5.4	x	0	xxxx	x	0	xxxx
0.001	7.0	x	0	xxxx	0	0	xxx
0.001	8.4	x	0	xxxx	x	x	xx
Distilled water		0	0	0	0	0	0

amount was never more than a trace. Practically all of the pentathionic acid was accounted for in the filtrates and spore masses. The results indicate that pentathionate ions are relatively stable in both the alkaline and the acid conditions; that is, they are stable under conditions when they are not toxic as well as under conditions when they are toxic.

The effect of H-ion concentration on the permeability of the wall of the fungus spore. The difference in the effect of pentathionate ions at different reactions is apparently due to the condition of the spore. Tests were made to determine whether the permeability of the cell walls to the pentathionate ions is affected by the reaction of the medium. Fungus material scraped from the fruiting surface of a colony of *Sclerotinia cinerea* was soaked for 6 hours in dilute solutions of pentathionic acid buffered at various reactions. The fungus material was removed from the acid, washed thoroughly with distilled water, and then tested with ammoniacal silver nitrate.

The reactions at which the tests were made and the results are shown in table 20. Nearly the same amount of color, due to the reaction of penta-

TABLE 20.—*The effect of H-ion reaction on the permeability of spore walls to pentathionate ions, determined by the precipitation of silver sulphide in the spores due to the reaction of ammoniacal silver nitrate and pentathionic acid*

Material tested	Concentration of $H_2S_5O_6$	pH	Color of inside of spores due to silver sulphide
Fungus material scraped from fruiting surface of *Sclerotinia cinerea* colonies on potato-dextrose agar	0.001 N.	2.0	Black
	" "	4.0	"
	" "	6.0	Brown-black
	" "	8.0	Black
	Check	6.0	Light brown

thionate ions with silver nitrate, was found in all the spores, regardless of the reaction of the solution in which they were soaked. It is concluded that the walls of spores of *Sclerotinia cinerea* are penetrated by pentathionate ions in both alkaline and acid reactions. The results of the tests indicate that the difference in the effects of pentathionates at pH 5.4 and at pH 7.0 is governed by the condition of the fungus tissue, probably the amphoteric substances, rather than by the condition of the pentathionate ions.

SUMMARY

1. The volatile product of sulphur is a vapor. The rate of vaporization is affected by temperature, a rise from 23 to 93° C. causing the rate to increase about 20,000 times. Sulphur vapor is not toxic to fungus spores, and the condensation products are toxic only after standing several hours.

2. Sulphur dioxide occurs only in traces in ground sulphur and is not toxic to spores of *Sclerotinia cinerea* in such concentrations. Light and high temperatures seem to favor the formation of the gas. As a transitory factor in the formation of pentathionic acid, sulphur dioxide probably plays an important rôle in the toxicity of sulphur fungicides.

3. Hydrogen sulphide does not occur in ground sulphur. Traces of the gas are produced from sulphur by the action of higher plants and fungi, but the gas is not toxic to fungi in such concentrations. Hydrogen sulphide is considered an active agent in the reduction of higher oxides of sulphur to pentathionic acid.

4. Pentathionic acid is associated with sulphur and is toxic to fungi in such concentrations. It is a natural oxidation product of sulphur, its formation being enhanced, however, by mild oxidizing agents and probably by hydrogen sulphide. It is not sufficiently volatile in dilute solutions to be toxic at a distance.

5. The toxic action at a distance is attributed to the combination of the following factors: the vaporization of sulphur and the oxidation of the condensed vapor that result in the formation of the toxic factor. Hydrogen sulphide is thought to be a contributing factor in these reactions.

6. The pentathionate ion is stable in weak acid and alkaline solutions, however, it is toxic only in acid solutions. The toxic action apparently is governed by the condition of the fungus rather than by the condition of the pentathionate ion.

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STUDIES ON THE DEVELOPMENT OF CROWN GALL, HAIRY ROOT, AND WOUND OVERGROWTHS IN TREATED SOIL[1, 2, 3]

A. J. Riker and W. M. Banfield

INTRODUCTION

The complexity of the problem of overgrowths on apple, with the attendant difficulty of accurate diagnosis, has been an obstacle to a clear understanding of the manner of infection, development, and symptoms of the diseases in this group. A number of different workers, beginning with Smith *et al.* (16), have pointed out that considerable variations occurred not only in the different overgrowths but also in various organisms that may be associated with certain of them. It appeared that the understanding of this complex problem would probably be advanced if methods of experimentation were improved to the point at which each of the several types of overgrowth could be induced at will without mixture with one another. The separate development under partially controlled conditions of crown gall and callus on nursery apple trees was accomplished by Riker and Keitt (12), and by Muncie (6). The earlier studies have been enlarged and extended by the present writers, who began this work at Madison, Wis., in the spring of 1927.

A number of different experiments have been performed to follow the development not only of crown gall, caused by *Phytomonas tumefaciens* (Smith and Town.) Com. S. A. B., but also of hairy root, caused by *Ph. rhizogenes* R. B. W. K. and S., and girdle knots. These trials were conducted under conditions where efforts were made to eliminate, as far as feasible in the field, the factors that might induce development of complicating overgrowths, and to induce only the particular one under consideration. A report of these studies, mentioned in an abstract (10), is given in the present paper.

METHODS

Several methods were employed that had varying refinements in the manner of regulating possible extraneous influences. These consisted primarily in growing apple grafts in the field (1) in beds of soil that, as far as records are available, had never grown a crop naturally infected with crown

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] These studies have been conducted in cooperation with the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] This work has been supported in part by a grant from the special research fund of the University of Wisconsin.

gall and (2) in similar soil that had been steamed. Various organisms were then introduced into certain beds. Further refinements of method in which portions of apple stems were enclosed in glass cylinders will be described in a later paper.

The soil selected for steaming in 1928 was a sandy loam of good fertility that had been employed for a number of years to grow truck crops. This soil was prepared by plowing and spading to a depth of 10 in. and was steamed according to the well-known method recently described by Johnson (4), which was sufficient to destroy bacteria of the crown-gall group. Immediately after steaming, 12-in. boards were placed edgewise around the beds so as to prevent washing in of surface water. Several of the beds were held as controls, while other individual beds were treated, respectively, with heavy suspensions of certain organisms under consideration. These suspesions were applied to the surface and worked down through the soil to a depth of 6 in. The control beds were sufficiently separated from the inoculated beds to make remote any chance contamination. The steamed beds were situated so that water could easily be supplied when desirable during dry summer weather. Six beds were employed for these studies, in which Wealthy apple grafts were planted at the rate of 400 grafts per bed. Although rather crowded, they grew very well during the time of the experiments. As the plants developed in these beds they were sprayed at various intervals with Bordeaux mixture to repel leaf hoppers and to prevent infection with various leaf-spot organisms, particularly that of apple scab. Soil of a similar type was employed for a repetition of the experiments in 1929. The treatment of the soil was similar except that the soil was not steamed. Since there was no available supply of water for the second series, the trees in this experiment suffered somewhat from drought, especially in the summer of 1930.

The cultures employed for treating the soil were as follows: Crown-gall, No. 2018, originally isolated from a large soft gall on apple and shown to be pathogenic on a number of plants, including apple; hairy-root, No. 2014, and No. T43-1e, originally isolated from woolly-knot overgrowths on apple and found able to induce hairy root on apple; a mixture of the crown-gall and hairy-root organisms, No. 2004, originally isolated from apple and that had produced symptoms of both crown gall and hairy root on apple, tomato, and tobacco; *Bacillus radiobacter,* Beij. and Van Deld., No. R 11, originally secured from the soil, was nonpathogenic on any of a series of plants tried. Studies of the morphology and physiology of these cultures are reported in a paper by Riker *et al.* (11) and by Wright *et al.* (17). Since the plants treated with *B. radiobacter* behaved like the controls, they are listed in later pages with the controls.

The pathogenicity of the cultures employed was determined in parallel studies, as shown in table 1. In each soil bed to which organisms were

TABLE 1.—*Results following control punctures and different types of inoculations below ground with crown-gall and hairy-root organisms on the scions and unions of Wealthy apple grafts grown in various soil beds*

Cultures used in soil beds and for inoculations	Treatment of grafts	Trees	Crown gall	Hairy root	Negative
Experiments on scions—results taken after growth of the trees during 1928 and 1929 in steamed soil[a]		Number	Number	Number	Number
Hairy root, No. 2014	Inoculated	39	0	33	6
Do	Punctured	20	0	0	20
Hairy root, No. T43–1e	Inoculated	30	0	16	14
Do	Punctured	20	0	0	20
Crown-gall and hairy-root mixture, No. 2004	Inoculated	36	33[b]	2	1
Do	Punctured	19	3	0	16
Crown gall, No. 2018	Inoculated	33	22	0	11
Do	Punctured	19	1	0	18
Control	Punctured	107	0	1	106
Experiments on unions—results after growth of the trees during 1929 and 1930 in natural soil					
Hairy root, No. 2014	Smeared[c]	94	0	5	89
Do	Inoculated	226	0	83	143
Do	Punctured	119	0	0	119
Control	Punctured	187	0	1	186

[a] The grafts were planted in steamed-soil beds to which various organisms, respectively, were added. As indicated, a portion of them were inoculated through punctures; the others were merely punctured, as explained in the text. The final results were taken at the end of the second growing season.

[b] Various degrees of hairy-root development were also shown by these overgrowths.

[c] Covered with bacteria.

applied, a number of trees were punctured, but not inoculated, and others were inoculated by means of punctures on the underground parts with the same organisms that had been earlier applied to the soil. The soil about the trees was momentarily moved aside from time to time as the season progressed, and the trees were examined to note the stages in the development of the various overgrowths. Except for addition of the bacteria, the

control beds received the same treatment. It is noteworthy that following the punctures in the soil treated with bacteria only an occasional infection occurred. Since these bacteria live in the soil for some time (8) and (1) and were isolated from the soil in these beds subsequent to this treatment, there was no doubt of their presence. Although the bacteria were present in the soil, the question is raised whether they were able to gain entrance into the punctures before these punctures were closed as infection courts. Similar results were found in other experiments recorded and discussed later in this paper.

This series of experiments in the field is open to the criticism that, while the possibility that the crown-gall and related organisms may be present as contaminants is reduced, especially at the beginning while the wounds are open infection courts, it has not been eliminated.

In the steamed soil these organisms were doubtless destroyed by the heat, if they were present. However, since both the steamed soil and natural soil had never, as far as is known, carried a crop susceptible to crown gall, it seems unlikely that this organism was present at the beginning of the experiments. The fact that the controls in both the steamed and natural soil remained nearly free from infection is evidence in favor of this view. However, there is always the possibility that some one of a number of possible agencies might have introduced an undesired organism of this group. The organism, if thus introduced, might move even through the soil, but such movement is so slow (3) that it is probably not an important factor, especially since the organisms under consideration are wound parasites. The greatest obstacle of all in this type of work is the possibility of introducing the causal bacteria upon the roots of the apple grafts. While various antiseptics may be applied to the surface of the seedling roots at the time the grafts are made, the writers, in a long series of unpublished trials, have not yet found any means of removing, with full confidence, all such organisms from the seedling roots. This is made extremely difficult because, at the time the seedling roots are harvested, a considerable number of the small lateral roots are either injured or broken off. Very frequently they die back to their origin, thus giving rise to the formation of small cavities. The present writers are unaware of any treatment that will destroy organisms in these cavities without injury to the main root, itself. The difficulty of growing seedling roots in any case, to say nothing of growing them under sterile conditions, prompted the present writers to seek other methods of approaching this problem. The method later employed involved placing portions of the stems of naturally growing plants under aseptic conditions and carrying on the experiments with these parts. The results of this work are reported in another paper.

DIFFERENTIATION OF VARIOUS TYPES OF DEVELOPMENT

The difficulty of making accurate diagnoses of the various malformations included in the complex under consideration has been considerably augmented because of the intergradations of the various symptoms and of the trouble in distinguishing one of several different stimulating agencies from the others. In the present studies, since certain particular causes of various overgrowths have been separated from one another as far as practicable, it is possible to clarify certain questions that have been raised concerning the identity of the different manifestations.

In the steamed soil beds in the field particular attention has been given to crown gall, hairy root, mixtures of crown gall and hairy root, and girdle knot. It is quite possible that the type of nonparasitic enlargements often encountered when different plants are grafted, such as those mentioned by Kostoff (5) and by Riker (9) is encountered from time to time on apple. A consideration of such overgrowths is not included in the present paper. This complication was intentionally avoided in most cases by inducing the different enlargements on the scion wood above the union.

The crown galls developed on these experimental plants, following inoculations with *Phytomonas tumefaciens*, conformed in appearance to the descriptions and illustrations that have already been given (12) and (6). The symptoms on these plants seem to correspond to those reported by Brown (2) and Siegler (14) as being induced by their "peach strain" of the crown-gall organism. The cultures used by the present writers were all isolated originally from apple.

Hairy root developed following inoculations with *Phytomonas rhizogenes* in accord with the description and illustrations by Riker *et al.* (11). Similar developments are described by Muncie and Suit (7).

Mixtures of the crown-gall and hairy-root organisms resulted in a series of overgrowths that showed crown galls with roots developing from various portions of the gall tissue. In some cases the roots came from the stem at the outer edge of the gall tissue and in others from the gall tissue proper. A rather complete intergradation between crown gall and hairy root was secured (Fig. 1, C, D). Some of the symptoms produced by *Phytomonas rhizogenes* and by a mixture of *Ph. tumefaciens* and *Ph. rhizogenes* suggested those found by Siegler (15) and Brown (2) following inoculations with their apple strain of the crown-gall organism.

Girdle knots (Fig. 1, B) were induced on the scions of the young apple trees after wrapping the stems several times around with copper wire at the time of planting. (Table 2). This was done in an effort to obtain the callus or wound-overgrowth type of manifestation that occurs following an interference with the downward flow of elaborated food from the upper portions of the plant because of failure at the graft union. At the same

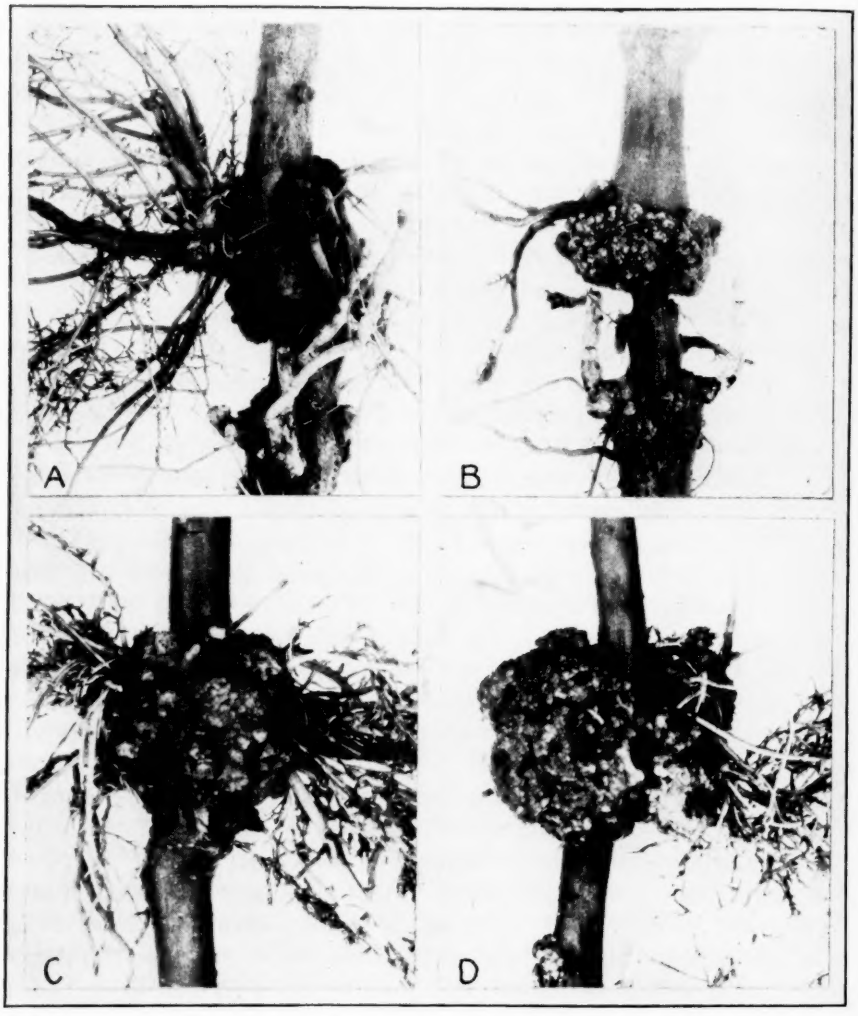

FIG. 1. Overgrowths developed in steamed-soil beds in the field after two seasons of growth. A. Noninfectious hairy root on the scion above a girdle of copper wire developed in the control bed. B. Noninfectious girdle knot on the scion above a girdle of copper wire developed in the control bed. C. and D. Overgrowths on the scion following inoculations with a mixture of the grown-gall and hairy-root organisms.

time, it was desired to avoid complicating this with other physiological influences, including incompatibility, which doubtless may occur at the union on apple. It is well known, (12), (6) and (13), that an imperfect union may provide a girdling influence. This girdling influence at the union may result from several factors, including (1) poor fit, (2) poor wrapping,

TABLE 2.—*The development of callus or wound overgrowths and noninfectious hairy root over two growing seasons on trees girdled with copper wire and planted in control beds and in beds containing crown-gall and hairy-root organisms, respectively*

Organisms in soil	Trees wrapped with wire	Girdle knots	Noninfectious hairy root	Possible infections[a]
	Number	Number	Number	Number
Results after growth during 1928 and 1929 in steamed soil				
Hairy root, No. 2014	34	16	10	4
Crown-gall and hairy-root mixture, No. 2004	37	15	15	4
Crown gall, No. 2018	38	14	12	1
Control	78	19	25	3
Results after growth during 1929 and 1930 in natural soil[b]				
Hairy root, No. 2014	244	107	30	3
Crown gall, No. 2018	143	15	7	3
Control[c]	161	11	13	0

[a] It is uncertain from their appearance whether these overgrowths were due to the girdle or to the combined action of the girdle and stimulating organisms.

[b] Dry weather retarded the development of these trees.

[c] At the end of the growing season of 1929 25 trees bearing large overgrowths at the girdle were removed.

(3) failure of parts of the union to form callus, and (4) the formation of a cork barrier between the scion and root. These girdle knots were induced by the writers in 1927 during experiments aimed at control, in which the unions were wrapped with string treated with antiseptics that prevented its decay. Hundreds of such knots were induced by different types of wrapping, which, due either to the nature of the material or to the method of application, did not decay before rapid secondary thickening of the apple stem took place. Although large girdle knots do not form in Wisconsin the first season from every tree so treated, those that survive this severe treatment over the second season nearly all show very considerable swelling and may even display masses of small roots coming from the region from $\frac{1}{4}$ to 1 in. above the constriction.

The character of these girdle knots induced by copper wire on the scion above the union is very similar to that earlier described (12) for wound overgrowths. A definite cortex surrounds the outer portion of the tissue after the knot has advanced beyond the fresh callus stage. This cortex may be quite irregular and show slight convolutions (Fig. 1, B). Not in-

frequently these convolutions may contain various soil-inhabiting bacteria. The interior of these girdle knots is commonly very hard and may be composed of tissue that is laid down in irregular directions, making them exceedingly difficult to cut with a knife. While typical girdle knots are easily recognized, there are phases of the later development of infectious hairy root that superficially come very close to some of the reactions following girdles. In these border-line cases it is not possible as yet to distinguish between infectious hairy root and callus knot with full certainty in the absence of cultural examinations. Doubtless varying degrees of mixtures of these two overgrowths occur commonly in nature. The question is raised as to whether the infectious hairy-root development, itself, may act as a partial girdle, so that this influence may be operating to supplement the action of the bacteria. Noninfectious hairy root (Fig. 1, A) often appeared on the trees in these studies, especially during the second year, above the tissue formed by the copper-wire girdle (Table 2). They more frequently arose from the stem tissue from $\frac{1}{4}$ to 1 in. above the point of constriction and resemble very closely the type of noninfectious hairy root that occurs commonly during some seasons on certain varieties of apple, such as Rome Beauty and Early Harvest.

The results from the experiments in both steamed and natural soil have been very consistent and have given so little mixture of the overgrowths studied that considerable confidence is placed in the outcome.

CALLUS NOT ORDINARILY AN OPEN INFECTION COURT

The manner of natural infection and its relation to callus tissue are the most critical considerations in the life histories of these organisms and may have very considerable importance in relation to the application of control measures. Working under ordinary conditions with the crown-gall organisms, Riker and Keitt (12) earlier reported that callus formed on apple grafts was "not commonly an open infection court" for the crown-gall organism. Rather similar results are reported by Siegler (15) for both his "peach" and "apple" strains.

The possibility of infection through callus tissue with several organisms was tried by the writers when the opportunity came to study this question under partially controlled conditions. A number of apple grafts were planted in different lots in beds treated, respectively, with the hairy-root organism, the crown-gall organism, and mixtures of these two, as well as in the control beds, as described earlier. Callus was induced upon the scions of young apple trees following wounds made during midseason. The wounds were produced by scraping the cortex with a scalpel until wood was exposed. These wounds were covered with soil and allowed to remain 3 weeks. After callus had formed certain of the trees in the con-

trol beds and beds to which bacteria were applied, respectively, were treated by covering the callus either with water or with cultures of the organism originally applied to the bed. Other trees were given the additional treatment of scraping the callus lightly with a needle after the water or bacteria, respectively, had been applied. Control inoculations with the same cultures through needle punctures produced positive reactions, which showed that the bacteria employed were in an active state. The results are shown in table 3. Here, it appears that a comparatively small percentage

TABLE 3.—*Callus produced by injuries in midseason not ordinarily an open infection court for the crown-gall and hairy-root organisms*[a]

Organism used in soil and for treatments	Trees treated	Reactions following treatments		
		Crown gall	Hairy root	Negative
	Number	Number	Number	Number
Callus covered with bacteria				
Hairy root, No. T43	27	0	0	27
Hairy root, No. 2014	31	0	1	30
Crown-gall and hairy-root mixture, No. 2004	28	1	0	27
Crown gall, No. 2018	27	0	0	27
Control	46	0	0	46
Callus covered with bacteria and scraped lightly				
Hairy root, No. T43	26	0	0	26
Hairy root, No. 2014	30	0	1	29
Crown-gall and hairy-root mixture, No. 2004	28	4[b]	1	23
Crown gall, No. 2018	27	1	0	26
Control	38	0	0	38

[a] The cortex below ground was scraped, on August 7, 1928, until wood was exposed. Callus had formed by August 29, when it was treated by covering with bacteria or by covering with bacteria and then scraping lightly. The final results were recorded in November, 1929.

[b] Various degrees of hairy root were also shown by these overgrowths.

of infections were secured where the callus was not wounded, which indicates that under these conditions the callus is not ordinarily an open infection court. These results are in conformity with those reported earlier (12)

and (15). Even in the cases where the callus was lightly scraped the percentage of infection was very low. Since puncture inoculations of the woody stems (Table 1) gave a higher percentage of positive results, it appears that under these conditions callus tissue was not so susceptible to infection as the punctured stem tissues. The mechanism of callous infection needs further study.

The callus formed above wire girdles also seemed to be not commonly an open infection court for any of these organisms. This was tested in experiments in which the grafts were wrapped with wire, as earlier described, and different lots, respectively, were planted in various inoculated-soil and control beds (Table 2). Four times during the development of the girdle knots, mass cultures of the same strains of bacteria used, respectively, to inoculate the soil of the different beds, were employed to smear the surface of the callus on the girdle knots in these beds. At the end of the season the girdle knots (Fig. 1, B) that were produced in the different inoculated beds were alike in all cases except as noted (Table 2) and were like those produced in the control beds. In addition to the formation of girdle knots, a considerable number of root developments of noninfectious character (Fig. 1, A) were also found either at the top of the girdle-knot tissue or on the stem less than a half inch above the enlargement. These apparently were developed irrespective of any bacteria and seemed to be natural responses of the trees to the interrupted downward passage of metabolic products. Since the bacteria were placed in the soil where they persisted for a long time before the grafts were planted and since bacteria were subsequently added to the surface of these overgrowths at four different times without wounding during the course of their development, the presence of the bacteria in the soil and on the surfaces of these knots during the course of their development in certain soil beds seems beyond reasonable question. Although a few doubtful cases occurred, the relative uniformity in the appearance of these developments, grown both in soil containing different stimulating organisms and in soil containing none, suggests that callus tissue formed under such circumstances is not ordinarily an open infection court for these organisms.

SUMMARY

1. Typical crown gall (*Phytomonas tumefaciens*), hairy root (*Ph. rhizogenes*), and girdle knots (copper wire), respectively, have been induced at will in both steamed and natural soil on nursery apple trees.

2. Inoculations with mixtures of the crown-gall and hairy-root organisms induced enlargements that showed both crown gall and hairy root, with a considerable range of the mixed characters.

3. Callus on Wealthy apple appears not ordinarily to be an open infection court for either of these bacteria.

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THE DEVELOPMENT OF CROWN GALL, HAIRY ROOT, AND WOUND OVERGROWTH IN GLASS CYLINDERS[1, 2, 3]

A. J. Riker, E. M. Hildebrand, and S. S. Ivanoff

INTRODUCTION

The production of crown gall, hairy root, and callus knots or wound overgrowths on nursery apple trees has been attempted under conditions designed to prevent the interference of any stimulating factors aside from the one under consideration. The need for studies of this kind has been recognized for a number of years and was emphasized by Siegler (5). More or less controlled conditions have been employed by several writers, including Riker and Banfield (2), who have reviewed this situation. Although, in these reports, the evidence against a mixture of stimulating agencies is strong, the different writers recognize the possibility that uncontrolled factors might perhaps have complicated some of the results. For this reason the studies reported in the present paper were undertaken. It was planned to induce the various enlargements being considered under conditions in which the possible presence of confusing influences would be eliminated to the utmost with the available technique.

EARLIER STUDIES INVOLVING ROOT TISSUE

The attempt was made in the earlier stages of the work to enclose the unions of piece-root apple grafts in aseptic chambers. This was done because, in work previously reported (Riker and Banfield, 2), the objection was raised that undesired influences may perhaps have operated under the partially controlled conditions. The very exacting requirements necessary for growing even a herbaceous plant under aseptic conditions made it seem undesirable to attempt to grow young apple trees where all microorganisms were excluded. For this reason it was decided to work only with parts of young apple trees.

Large flasks of 3-liter capacity were employed as containers for growing piece-root apple grafts. In some cases these flasks were filled with sterilized white sand and nutrient solution and in others with steam-sterilized soil. The soil was autoclaved in small quantities for 5 hours at 15 pounds' pres-

[1] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

[2] These studies have been conducted in cooperation between the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the University of Wisconsin.

[3] This work has been supported in part by a grant from the special research fund of the University of Wisconsin.

sure on each of 3 successive days. Aeration tubes to the top and bottom of the flask were provided through rubber corks. Piece-root apple grafts were prepared under aseptic conditions from surface-sterilized material and were inserted in these flasks with two buds extending through the rubber at the top. The seal between flasks, rubber stopper, graft, and glass tubes was closed by a layer of sterilized vaseline, 5 mm. deep. All the manipulations in these experiments were designed to exclude the entrance of microorganisms. For example, air filtered through sterile cotton was supplied as needed through the aeration tubes. The water employed to maintain moisture was distilled and sterilized. Inoculations were made on the piece-root grafts either at the time the experiment was set up or after the graft had become established by opening the chamber in a transfer room.

The results in the large flasks were rather disappointing for two reasons. First, under the conditions of the experiment, the piece-root apple grafts did not make sufficiently rapid growth to enable the various enlargements to develop in a manner parallel to that found in the field. Second, through a fundamental difficulty in the technique of surface sterilizing the roots from which the grafts were made, as explained later, the writers were unable to accomplish complete disinfection; consequently, the detailed results are omitted.

Glass cylinders were employed in parallel studies. These were fitted about the unions of piece-root apple grafts and planted in large containers, in the usual manner, in the greenhouse. In these earlier studies attention was directed particularly to the union between the scion and the root because this is the position at which the largest percentage of malformations occurs upon trees grown from piece-root apple grafts in the nursery. These cylinders are described in a later section of this paper. Grafts were prepared from surface-sterilized scions and roots and inserted in the sterilized glass cylinders under aseptic conditions. The tops and bottoms of the cylinders were closed with sterile sections of rubber stoppers and sterile vaseline. Waterproof rubber caps were provided over the ends of the cylinders before the grafts were planted in either large pots or soil cans in the greenhouse. In some cases the grafts on which the cylinders were mounted were planted so that the cylinders were beneath the soil; in others the cylinders were placed at the surface of the soil. The glass walls made possible frequent observations upon the developments that were taking place.

The results secured in the cylinders mounted on the unions of piece-root apple grafts were disappointing for the same reasons that have been given for the grafts grown in 3-liter flasks. The growth was not sufficiently vigorous to give results parallel to those secured in the field and it was not found possible to exclude all the undesired microorganisms. The greatest difficulty was experienced with various fungi that appeared from time to time

in many of the cylinders. Although the writers were not particularly concerned about the influence of these fungi upon the development of overgrowths, their appearance suggested that the technique employed was not sufficiently exacting, and results are not reported from such experiments.

The failure to secure aseptic conditions in the flasks and glass cylinders appeared to be primarily due to the difficulty in surface sterilizing the root stocks that were employed in making the grafts. This was due not to the inefficiency of the surface disinfectants employed but to the difficulty of reaching all the microorganisms without at the same time producing so much injury on the roots that they would not grow. The fundamental difficulty appeared following a closer examination of these seedling root stocks. It was evident that when the seedling roots were removed from the field a considerable number of very small rootlets were broken off during the process of pulling. Microorganisms entered the vascular tissue of these very small rootlets and penetrated the roots for a considerable distance. Any antiseptic, sufficiently strong to penetrate these tiny openings and kill the microorganisms present, was also strong enough to produce very severe injury to the root itself. For this reason such work involving seedling roots did not appear promising.

The details of the experiments with the cylinders placed about the unions are omitted because of these difficulties. However, these and subsequent experiments threw interesting light on the effect of adhesive tape on the development of callus from the cut surfaces and on the production of callus-like growth from the lenticels.

The effect of adhesive tape on the formation of callus at the union between scions and roots was observed in some of the experiments in these glass cylinders mounted about the unions of piece-root apple grafts. Where the union was only partially covered with adhesive tape, it was observed that an excess of callus tissue pushed out in some cases where the grafts were growing more or less vigorously, between the strips of adhesive tape (Fig. 1, A). This very strongly suggests that adhesive tape may act as a mechanical agency to prevent the lateral extension of excess callus and to force this callus tissue back through any openings that may occur between the cut surfaces of the graft. This appears to have the effect of preventing the formation of excess callus on the surface and of increasing the opportunity for good union between the scion and the root. This in itself would probably reduce the amount of girdling action due to a failure of union.

The formation of a considerable amount of tissue superficially resembling callus was also observed to appear from the lenticels of both the scions and the roots, which were enclosed in these glass cylinders and in which the humidity was maintained at a very high level. Küster (1) has discussed this subject. This type of development was rather confusing when the early

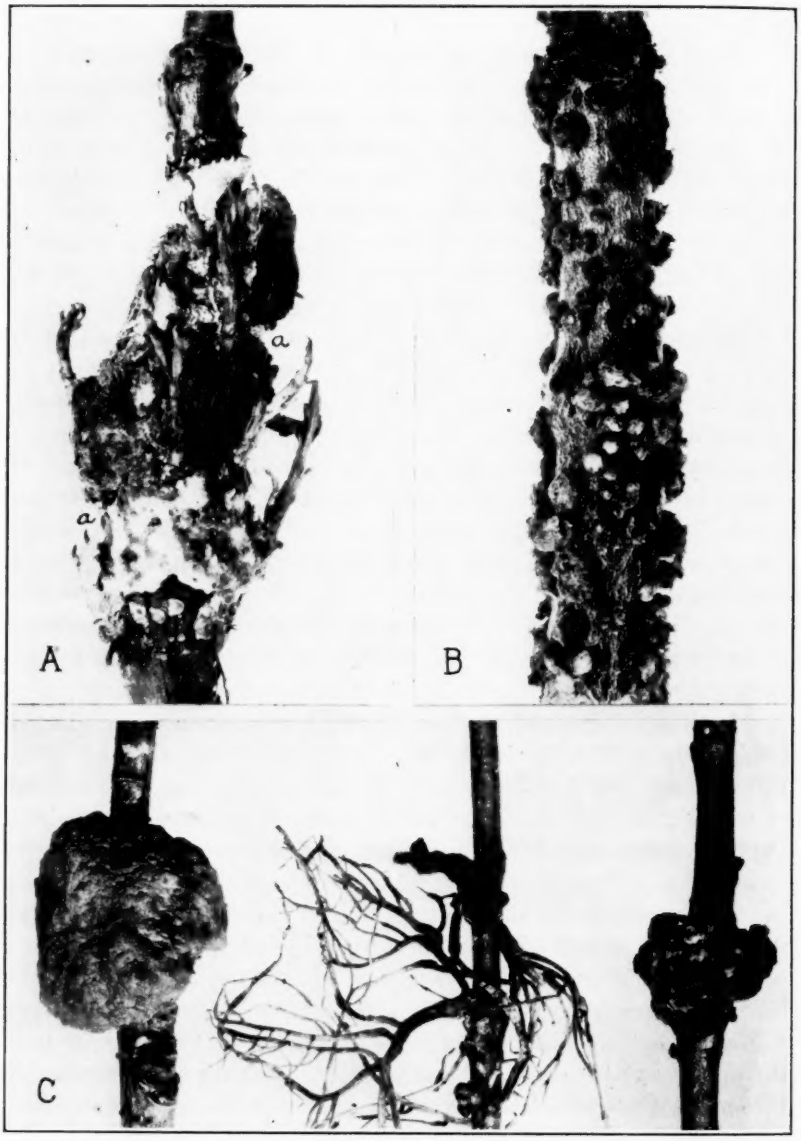

FIG. 1. Overgrowths on apple induced in glass cylinders. A. Excess callus induced at the union of a Wealthy apple graft that was only partially covered with ⅛-in.-wide adhesive tape. Depressions in the callus at *a* show the earlier positions of the adhesive tape. B. Developments from the lenticels of a Wealthy apple stem kept in saturated air. C. Maximum developments on the stems of Yellow Transparent apple trees following inoculations with the crown-gall organism and with the hairy-root organism and after girdling with copper wire, from left to right, respectively. The crown gall was shaped against the glass wall of the cylinder.

stages of the development of crown gall and hairy root were considered and presented the problem of satisfactory aeration within these chambers. An illustration of this type of growth from the lenticels of scion tissue may be seen in figure 1, B.

PRODUCTION OF OVERGROWTHS ON STEM TISSUE

Glass cylinders placed on the aboveground parts of nursery apple trees grown under natural conditions promised a means to avoid the difficulties that were encountered on seedling roots.

In the summer of 1928 and 1929 sterile glass or galvanized-iron chambers were employed. These chambers could be placed about portions of actively growing apple stems and so sealed as to allow the surface of the enclosed portion of the stem to be sterilized and to remain under aseptic conditions. This method is an adaptation of that employed by a number of plant pathologists to provide moist chambers about parts of plants. The cylinders used were usually 3 in. long by $1\frac{1}{4}$ to $1\frac{1}{2}$ in. in diameter. The cylinders were slipped over the tops of rapidly growing shoots of the current year's growth to the locations selected for special treatment. After being brought to position they were washed with a cloth wet with an antiseptic. The portion of the stem was prepared by removing all the leaves and also by washing it with an antiseptic, such as silver nitrate, 1 to 1,000. The lower ends of these cylinders were closed with comparatively thin sections of sterilized rubber stoppers of the desired thickness and diameter and with a tapering hole of suitable size in the center to permit expansion by growth of the stem without development of enough pressure to cause girdling. To insure perfect fit the rubber stopper at the lower end of the cylinder was covered in most cases with a layer of sterilized vaseline applied in a liquid state. For cylinders that were entirely closed, another stopper was similarly applied at the top. Aeration was supplied in some cases through sterile cotton in tubes extending through the upper stoppers. In some cases waxed-paper covers were also provided to prevent wetting by rain. Commonly, final sterilization was made by filling the entire cylinder with silver nitrate, 1 to 1,000, for 5 minutes. After the antiseptic was drained off, the inoculations were performed before the top was closed. Several variations of this technique were employed, which included filling some of the cylinders with soil that had been sterilized in small quantities for 5 hours on each of 3 successive days, at 15 pounds' pressure, and others of the cylinders with sphagnum that had been similarly sterilized. The glass cylinders were used almost exclusively in preference to similar cylinders made of galvanized iron because one could make observations more easily through the glass. A limited number of the cylinders were prepared with a lateral T extension, $\frac{1}{2}$ in. in diameter by 1 in. in length. This was closed with a rubber stopper

that could easily be removed to enable the operator to reach the stem inside without disturbing the upper or lower part. While this T extension was very useful for certain experiments it was found unnecessary for most of the trials, which did not require further attention after they were once started.

The efficiency of the method was tested by cultural examinations of the controls and overgrowths at the end of the experiment, which showed that many of them either were sterile of microorganisms or contained only the organisms introduced. A few of them became contaminated with fungi, but none except those inoculated showed any bacteria resembling in colony characters those in the crown-gall group. In these aseptic chambers placed about small portions of stems of apple trees, the various manifestations of crown gall, hairy root, and callus or wound overgrowth were induced without confusion of results due to undesired microorganisms. Although some difficulty was encountered in the 1929 and 1930 work, because of water of condensation in the chambers, this was practically overcome by providing satisfactory aeration through sterile cotton.

Crown gall was produced in these sterile chambers, which had the characters as described on apple by Riker and Keitt (4).

Hairy root, as described by Riker *et al.* (3), was likewise produced under these conditions, although the roots that developed following inoculation were frequently more fleshy, probably because of the relatively high humidity within the chambers. In many cases considerable root development was produced in cylinders that were exposed to full sunlight.

The callus, produced as the result of girdling, developed in a manner similar to that in the soil in the early stages. However, in the comparatively short time covered by the experiments with the sterile chambers in the 1929 and 1930 trials, the callus never reached the stage at which large woody knots were found. In some cases large soft callus knots were induced; in others, small hard callus knots were developed. The difference seemed to depend largely upon the relative humidity.

A summary of these 1928 and 1929 studies in aseptic chambers shows that negative results were secured in a number of cases because the interiors of the chambers were either too wet or too dry. Out of 70 trees inoculated with *Phytomonas tumefaciens* (Smith and Town.) Com. S. A. B., 16 showed well-developed crown galls and none showed hairy root. Out of 90 trees inoculated with *Ph. rhizogenes* R. B. W. K. and S., 43 showed well-developed hairy root and none showed crown gall. Out of 70 punctured control trees none showed any overgrowths, while of 20 girdled trees all showed more or less conspicuous girdle knots. In all the chambers with very high relative humidity there was a noticeable development of tissue from the lenticels (Fig. 1, B).

Repetition and extension of the experiments of 1928 and 1929 were carried out in 1930. Advantage was taken of the experience gained in earlier years, so that considerably better results were secured.

Several modifications of the methods employed deserve mention. The experimental trees were 2 years old. The cylinders were usually placed on the lateral twigs that were produced the year before, which brought the cylinders several feet above the surface of the ground. The cylinders were closed both top and bottom with sections of rubber stoppers but were not sealed with vaseline. Chemical treatment of the surface was made for 15 minutes with a 20 per cent solution of a commercial sodium hypochlorite preparation (Bacilli Kill, containing 3.5 per cent sodium hypochlorite). When necessary, the surface of the apple stem was rubbed to remove air pockets. After this treatment the stem was washed with sterile distilled water for the same time. Inoculations were made with a scalpel at three points on the stem. The interior of the cylinder was then loosely filled with sterilized sphagnum that was moistened with a 5 per cent solution of the sodium hypochlorite preparation and closed with a section of rubber stopper. From time to time as it was needed, the sphagnum was moistened with additional quantities of this 5 per cent solution. Observations were easily made when desired by uncovering a hole in the upper section of rubber stopper, by inserting a sterile glass rod through this hole, and by pushing the sphagnum to one side. The concentration of the sodium hypochlorite solution was such that no noticeable injury was produced on the apple tissue, and no difficulty was experienced with contaminating bacteria or fungi.

The cultures employed in these studies were all the progeny of single-cell isolations. Crown-gall culture, T–5, and hairy-root culture, C–1, were employed. Their bacteriological characters have been given by Riker *et al.* (3).

A series of treatments were made on the stems enclosed in these cylinders during May, June, July, and August at Topeka, Kans. as shown in table 1. The stems were treated by wounding, by inoculating with the crown-gall organism, with the hairy-root organism, and with mixtures of both, and by girdling with copper wire. The responses made toward the end of the series were not sufficiently far advanced by the end of the growing season to be definite. This situation was probably due to the failure of the trees to continue growth. The drought of 1930 probably augmented this situation considerably. The detailed environmental conditions covering the period of this work will be reported in a later paper.

The incubation periods for the different reactions following various treatments, respectively, are given in table 1. The amount of radial extension necessary for accurate diagnosis varied with the different overgrowths.

The character of the reactions secured in this series of experiments is quite clear and typical (Fig. 1, C). Crown gall, like that described by

TABLE 1.—*Incubation periods required for the development of various overgrowths in glass cylinders on the stems of Yellow Transparent apple trees following treatments at different times*

Treatments	Total stems treated	Total stems positive	Minimum incubation periods following treatments on:					
			May 26	June 9	June 23	July 7	July 21	Aug. 4
	Number	*Number*	*Weeks*	*Weeks*	*Weeks*	*Weeks*	*Weeks*	*Weeks*
Wounds[a]	50	45	8	7	6	6	8	?[b]
Inoculations—								
Phytomonas tumefaciens[c]	60	34	8	7	8	8	?	?
Ph. rhizogenes[d]	60	34	8/12	4/10	5/10	8/?	?	?
Mixture[e]	30	18	8/12	7/10	6/10	8/?	?	?
Wire girdle[f]	30	13	5	6	8	?	?	?

[a] The time is that required for wounds to cork over.

[b] The interrogation points show that the typical response was not secured before November 15, the last day records were taken.

[c] The single-cell culture, T–5, was employed. The time is that required for a 4-mm. radial extension of gall tissue.

[d] The single-cell culture, C–1, was employed. The time required for a 3-mm. radial extension of tissue and the formation of root primordia is expressed as the numerator. That required for roots over 6 mm. long is given as the denominator.

[e] A mixture of cultures T–5 and C–1 was employed. The time is given as for *Phytomonas rhizogenes*.

[f] The time is that required for a 2-mm. radial extension. Responses failed to develop in some cases, especially later in the season, because the stems stopped growing.

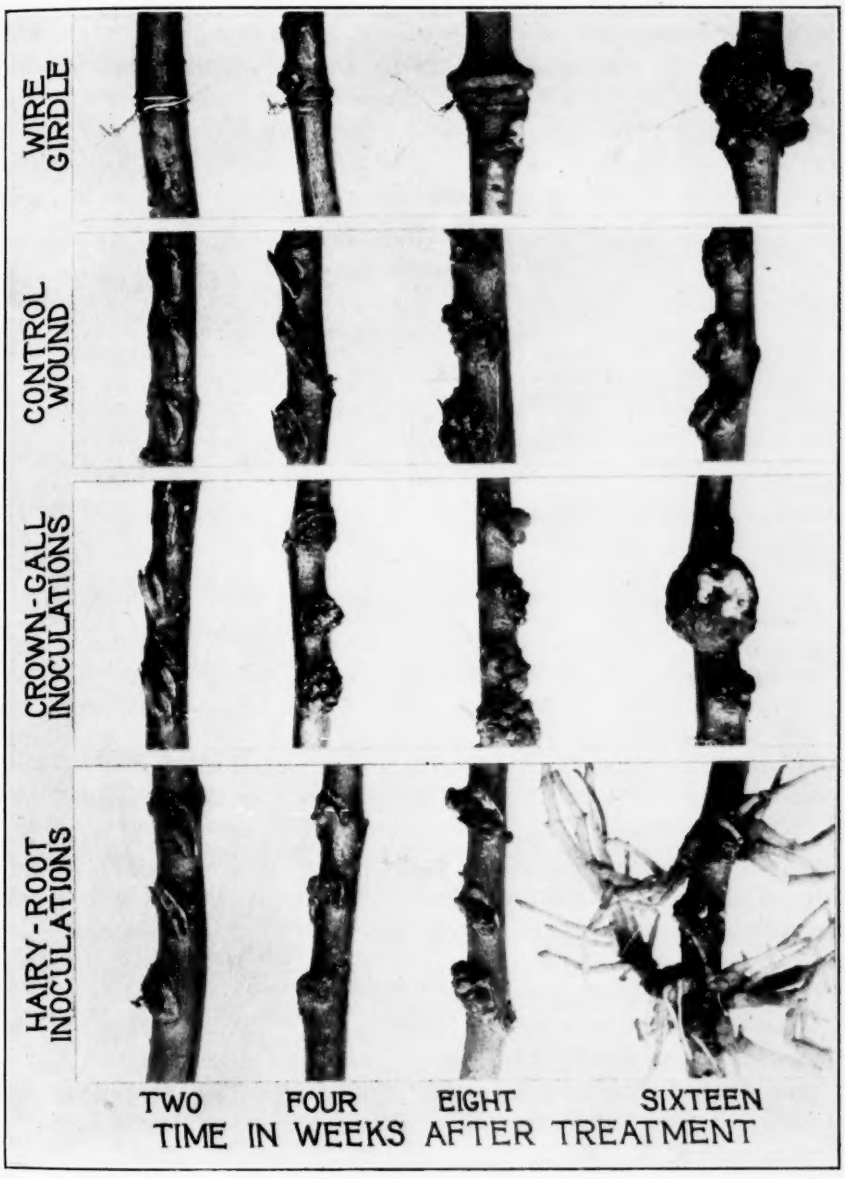

FIG. 2. Typical developments following various treatments at different periods of time on Yellow Transparent apple stems enclosed in glass cylinders.

Riker and Keitt (4), was induced following inoculations with *Phytomonas tumefaciens*. Hairy root, as described by Riker *et al.* (3), was induced following inoculations with *Ph. rhizogenes*. Inoculations with mixtures of these two showed various grades of mixed characters (Riker and Banfield, 2). The reactions to wire girdles showed callus knots or wound overgrowths that were quite characteristic (Riker and Banfield, 2). A summary of the inoculations made in May and June, 1930, which had satisfactory incubation periods are shown in table 2. The various stages in the development of the more important types of reactions are shown in figure 2. Cultural examination of representative enlargements showed them to contain either no microorganisms or only those which had been inserted.

TABLE 2.—*Development of various overgrowths in glass cylinders on the stems of Yellow Transparent apple trees*

Treatments	Number of stems treated	Stems showing number of				
		Only wound response	Crown gall	Hairy root	Mixture	Wire girdle
Wounds	30	30	0	0	0	0
Inoculations[a]—						
Crown-gall organism	30	6	24	0	0	0
Hairy-root organism	30	3	0	27	0	0
Mixture of these organisms	15	1	0	0	14	0
Wire girdle	15	0	0	0	0	13

[a] The responses to the crown-gall organism were counted only when the galls had a radial extension of over 4 mm. Those to the hairy-root organism had roots over 6 mm. long. Inoculations were made during May and June. The final results were taken November 15.

SUMMARY

These studies are interpreted as showing that crown gall, hairy root, and callus knot or wound overgrowth have distinct characteristics that may be induced by different agencies under rigidly controlled conditions. The methods employed have reduced to a minimum the possibility of a mixture of causal agents.

The best responses were secured following treatments made before the first of July.

Incubation periods of at least 2 months were found desirable.

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THE PATHOGENICITY OF FUSARIUM CONGLUTINANS WR. AT LOW SOIL TEMPERATURES[1, 2]

L. M. Blank

For some years the idea has prevailed that 17° C. is the minimum soil temperature at which cabbage yellows becomes manifest in susceptible host material. This conception has arisen as a result of the work of Gilman,[3] Tisdale,[4] and Tims.[5]

In the studies reported by Gilman seedlings were grown in flats of infested soil in greenhouses where the temperatures were controlled only within wide limits. In several trials pots of seedlings were placed in glass chambers in an attempt to control the soil temperature more closely. Gilman concluded that soil temperatures of 12° to 16° C. prevented the occurrence of the disease and that characteristic symptoms occurred at temperatures of 17° or above. Exceptions to these conclusions, however, were noted occasionally at the lower temperatures.

Tisdale, using the Wisconsin soil-temperature tanks, studied somewhat more carefully the lower part of the temperature range of the fungus. His conclusions were in accord with those of Gilman, that "17° C. is approximately the low critical soil temperature for the disease occurrence." With highly susceptible strains of cabbage no disease occurred at soil temperatures of 14° and 15°.

Somewhat later Tims carried on studies with temperature-control equipment similar to that used by Tisdale. His results in relation to the occurrence of the disease at the lower part of the temperature range agreed with those of the previous workers. In no trial did the disease develop at soil temperatures as low as 15° C. and only a comparatively small percentage developed at 18°. Tims, however, does note that "the yellows disease is found in the roots of many plants grown at a soil temperature of 15° C."

In connection with other studies on cabbage yellows the writer has noted the disease to appear and progress in a destructive manner at what had been

[1] This study is the result of cooperative investigations between the Department of Plant Pathology, University of Wisconsin, and the Division of Horticultural Crops and Diseases, Bureau of Plant Industry.

[2] Published with the approval of the Director of the Wisconsin Agricultural Experiment Station and the Chief of the Bureau of Plant Industry.

[3] Gilman, J. C. Cabbage yellows and the relation of temperature to its occurrence. Ann. Missouri Bot. Gard. 3: 25–84. 1916.

[4] Tisdale, W. B. Influence of soil temperature and soil moisture upon the Fusarium disease in cabbage seedlings. Jour. Agr. Res. 24: 55–86. 1923.

[5] Tims, E. C. The influence of soil temperature and soil moisture on the development of yellows in cabbage seedlings. Jour. Agr. Res. 33: 971–992. 1926.

considered the lower end of its temperature range. Therefore, experiments were initiated to determine more clearly the minimum soil temperature for the development of yellows in cabbage and other subspecies of *Brassica oleracea* L.

The experiments were conducted during the months of December and January for it was necessary that the soil temperatures be controlled as closely as possible. The trials were therefore carried on only during these months for three winters. The seedlings used in the several experiments were grown in clean soil for a month or more in the greenhouse at temperatures ranging from 15° to 20° C. The soil into which the seedlings were transplanted had been inoculated several years previously with cultures of the yellows organism and was known to be thoroughly infested. The cans containing these seedlings were immediately placed in the Wisconsin soil-temperature tanks and the desired temperatures attained by running a continuous stream of cold water into the tanks, or by use of an electric heating unit, if higher temperatures were desired. The air temperature in the greenhouse varied around 15° C., without excessive increases during the middle of the day. To further control the soil temperature in the cans about an inch and a half of ground cork was placed on the surface of the soil following transplantation of the seedlings. Standardized thermometers were placed in the cans of soil and in the water in each tank. The soil moisture in the cans of seedlings was maintained at a condition favorable for seedling development. The seedlings used in the several trials were of commercial lots and included three varieties of cabbage, three of kale, and one each of collard, cauliflower, kohl-rabi, and Brussels sprouts. The seed lots did not include any of the yellows-resistant selections but represented commercial varieties in common use. The varieties used in the trials were Moss Curled Dwarf, Thousand-headed, and Siberian kale; New Snowball cauliflower; Georgia collard; White Vienna kohl-rabi; Long Island Improved Brussels sprouts; and Early Jersey Wakefield, Early Flat Dutch, and Glory of Enkhuizen cabbage.

The preliminary trials were conducted mainly with subspecies of *Brassica oleracea* other than cabbage. In trial 1 the three kale varieties were studied at 2-degree intervals of soil temperature, the range being from 12° to 20° C. Previous workers[6] have noted the difference in susceptibility to yellows displayed by these three kale varieties, Moss Curled Dwarf being the most susceptible and Siberian kale, the least. In the temperature tanks similar results were noted. The yellows was evident in the Moss Curled Dwarf at 14° and above, with the disease severe enough to result in dead

[6] Walker, J. C., and F. L. Wellman. A survey of the resistance of sub-species of *Brassica oleracea* to yellows (Fusarium conglutinans). Jour. Agr. Res. 37: 233–241. 1928.

seedlings at 16°. Thousand-headed kale seedlings showed symptoms of the disease at 16° and dead plants were present in the 18° C. tank. No disease was evident in the Siberian kale seedlings at 21° C., the highest temperature studied. Approximately 45 seedlings of each variety were examined at each of the temperatures.

The next test was made the following winter and included seedlings of Moss Curled Dwarf and Thousand-headed kale, kohl-rabi, and cauliflower. The temperatures at which the seedling reaction was studied were 14°, 16°, 17°, and 19° C. After 33 days' exposure to these temperatures the final notes were recorded and it was found that the disease symptoms were manifest in both kale varieties and in kohl-rabi at 14° and in the cauliflower seedlings at 16°. Seedlings killed by the disease were present in the 16° tank in all lots except cauliflower, where they first appeared in the 19° tank. In this trial approximately 24 seedlings of each lot were considered at each of the 4 soil temperatures.

The evidence gained in the foregoing trials indicated rather conclusively that the minimum temperature for the occurrence of the yellows was below 17° C. Therefore in another final trial the soil temperatures were controlled with extreme care. Two of the tanks were regulated to give a soil temperature of 12° to 13°, 2 at 14° to 15°, and 1 at 16° to 17°. The soil temperature in the tanks was further controlled by the use of about 1½ in. of ground cork, placed on the surface of the soil in the cans following transplantation of the seedlings. Thermometers, inserted into the soil about 1½ in., were read several times daily and the temperatures recorded. Over the 42 days' duration of the experiment 92 soil-temperature readings were recorded for each tank and the average temperature prevailing in each tank was computed from these readings. The maximum soil temperatures recorded in the various tanks were 14°, 14.3°, 15.8°, 14.8°, and 17.3° C., respectively. In only one case did the maximum soil temperature exceed the point previously considered as the lower critical temperature for the occurrence of the disease. We may consider the four lowest temperature tanks as having an average soil temperature well below 17° C., as indicated in the table, and also with a maximum temperature below the previously reported critical temperature.

Varieties used included three of cabbage, and one each of kale, cauliflower, collard, kohl-rabi, and Brussels sprouts. The seedlings, which had been grown in yellows-free soil, at an air temperature of about 15° C., were transplanted when 34 days old into cans of yellows-infested soil and immediately placed in the tanks. Thirteen seedlings of each lot were studied in each of the temperature tanks. The results of this test are given in table 1.

The symptoms of the disease appeared in the collard seedlings in tank V 12 days after transplanting them into the yellows-infested soil. After 18

TABLE 1.—*Occurrence of yellows in crucifer seedlings exposed for 42 days at various soil temperatures*[a]

Tank No.	Average soil temperature	Number of seedlings diseased								Total plants diseased
		Cauliflower	Collard	Brussels sprouts	Kale	Kohlrabi	Cabbage			
							Jersey Wakefield	Early Flat Dutch	Glory of Enkhuizen	
	°C.									
I	12.1	0	0	0	2	0	1	2	0	5
II	12.4	1	1	0	2	1	1	2	2	10
III	14.4	4	4	1	3	1	6	7	6	32
IV	14.6	1	3	2	2	1	6	9	5	29
V	16.5	4	10	7	7	7	12	10	9	66

[a] Thirteen seedlings of each lot at each temperature.

days it was evident in seedlings of the remaining lots in the tank; at the next lower temperatures (tanks III and IV), the disease was first noted after 20 days and in tanks I and II after 25 days. At the lowest temperatures (tanks I and II), the disease was manifest in 5 and 10 of the total number of seedlings, respectively, after 42 days, as shown in the above table. The disease symptoms at these lowest temperatures were slight, usually evident on but one leaf per plant. In tanks I and II the organism was recovered from kale and from two varieties of cabbage; in tanks III, IV, and V it was isolated from each of the 8 lots of seedlings.

The amount and severity of the disease were directly dependent on the soil temperature, as has been noted by the previous workers. It may be observed that the amount of disease in tank IV was slightly less than that in tank III, although the average soil temperature was slightly higher. This slight discrepancy, however, may be explained by the fact that the maximum temperature recorded in tank III was higher than that of tank IV and that, although the average temperature was lower, the higher temperature, prevalent in tank III for a few hours, may have been sufficient to stimulate the development of the disease in tank III. The severity of the disease was sufficient in tank V to result in the death of seedlings of all varieties, and, in some lots, to the extent of half or more of the total number of seedlings. An occasional seedling was killed by the yellows organism in tanks III and IV, while at the lowest temperatures (tanks I and II) the seedlings that showed symptoms of the disease were not killed.

From the foregoing results it is concluded that the yellows of crucifers may develop at a temperature considerably below that previously reported. Since the disease was apparent at the lowest soil temperature studied in the present trial the low critical temperature for the development of the disease has not been determined, but it is thought to be close to 12° C. The writer, however, is convinced that cabbage yellows may become manifest at a soil temperature of 12° to 13° C., which is considerably lower than the heretofore-reported soil temperature of 17°.

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BOOK REVIEW

Miller, Edwin C. *Plant Physiology.* vii–xxiv + 900 pp., 38 figs. Edition I, McGraw-Hill Book Company, Inc., New York and London. 1931. Price, $7.00.

Here at last we have what is probably the most comprehensive treatise on plant physiology in the English language. A work of this character, treating the subject as it does from the standpoint of the basic importance of the plant cell; of solutions and membranes in relation to the plant cell; the intimate and complex relation of the plant and its various organs to their environment; the various vital processes peculiar to the growing plant, such as photosynthesis, processes of digestion, translocation of materials, respiration, and growth, will at once prove a valuable adjunct to not only the working library of the plant physiologist but also that of the plant pathologist, the agronomist, and the horticulturist.

The author's long service as a teacher and his extensive experience in botanical research has given him an unusual opportunity to acquaint himself with the now voluminous literature on the subject. This familiarity with his field and the published matter bearing upon it is plainly evident to him who takes the time to thumb leisurely the 850 pages of meaty text.

That ecologist or plant pathologist or other plant-science student, be he teacher or investigator, cannot review but with profit to his teaching or his research the first and second chapters of this text on plant physiology. It will, no doubt, seem to some that the author has devoted unnecessary space to a more or less elementary presentation of definitions and facts long known and not in the strict sense of the word a part of the lingo or thought of plant physiology. Yet, we must admit that an understanding of the plant cell—its structure as well as its behavior—is fundamental to our grasp and interpretation of facts disclosed by our research on the normal and abnormal physiologic behavior of plants.

On reading the text of Miller's Plant Physiology some may experience a sense of disappointment on finding that too little of it is substantially Miller's. One should not, however, overlook the fact that the evaluation and interpretation of the scientific worth of the more than 3,150 papers referred to in the text are Miller's. Moreover, it is probable that hundreds of papers not referred to at all were subject to his judgment.

One reader and critic of the work has said of it: "It is a remarkable treatise on the subject of plant physiology; remarkable for what it contains and for what it omits, and notable among the things omitted is the author's own contribution to the subject." Evidently, our critic had not taken cognizance of the fact that there are here and there throughout the text as many as 58 references to work done and published by Doctor Miller,

himself, since 1910. The author's most important contribution to plant physiology, quantitatively and probably qualitatively, has come through his investigation of the duty of water in plant metabolism and the transpiration phenomena of various crop plants. And yet, notwithstanding this fact, he has not devoted a disproportionate amount of space to the chapters bearing directly on these aspects of the subject in hand.

The treatment of the following chapters: The Roots of Plants; The Intake of Water by Plants; The Elements Absorbed by the Plant; The Loss of Water from Plants, and The Translocation of Materials in Plants could have been improved had the author devoted more space to the consideration of factors, vital and other, that inhibit the proper functioning of plants and plant organs. On the other hand, there is sufficient reference to such factors as soil-infesting and plant-disease organisms to indicate to the plant pathologist and the agronomist the unmistakable importance, if not necessity, of their acquiring a more thorough working knowledge of the relationship of such organisms to crop ecology.

We need to know much more than we do about the relation of root-invading organisms to root development of the host, their effect on the metabolism and water requirements of the host, their effect on the water-intake capacity of the invaded plants, and their ability to produce fruit or seed. We need to know more than is known about the effect of plant rusts, mildews, smuts, and the more obscure parasitic organisms on the quality as well as quantity of fruit or seed. As an important aid to such research we have Miller's Plant Physiology pointing the way, replete with references to a voluminous literature the digestion of which cannot but prove invaluable to those engaging in crop-ecological or phytopathological research.

The author's comprehensive review of the literature on the intake of water by plants, in which he devotes special attention to (1) sources of the plant's water supply, (2) wilting coefficient and factors influencing it, (3) water-supplying power of the soil and time of wilting, and (4) entrance of water into the root, should prove helpful to the student of root and foot rots of various crop plants and to those engaged in the investigation of the relation of rusts and other parasitic fungi to the water requirements and total yield of plants.

The author's thoroughgoing treatment of the subject matter of Chapters VIII, IX, and X, entitled The Formation of Carbohydrates by the Green Plant, The Nitrogen Metabolism of the Green Plant, and The Fat Metabolism of the Green Plant, cannot but prove helpful to teacher and investigator alike. We have long needed just this sort of valuable service by one who is at once the accomplished, exacting teacher and research worker in plant physiology.

It is all very well for the plant pathologist to inform himself on the details of the physiology of parasitism, the various steps and stages in the life history of his smuts and rusts and mildews and root-rot organisms, the symptoms and etiology of this or that plant disease, but it is quite as fundamentally important that he fully acquaint himself with the structure, growth, and physiologic behavior of the disease-free host. This done, he can the more intelligently interpret the results following invasion of the host by this or that parasitic organism or the abnormal behavior of plants when subjected to conditions of mineral deficiency or meteorologic upset.

The host of this or that pathogen is, for the period of active parasitism of the pathogen, its medium. But the medium, unlike potato agar or some other kind of agar or gel, is a vital thing played upon by light, heat, humidity, salts in solution, and a variety of other factors. It receives, digests, assimilates, elaborates, grows, breaks down under catalytic agents, and reproduces its kind. All of these processes are ably presented to the reader by our author and thus made available to the investigator in the fields of plant physiology and phytopathology. One can read between and within the printed lines of these chapters the fact that the ultimate expression of the parasitic consequences of fungous invasion or bacterial infection must of necessity depend upon the status of the medium. The medium is sensitive, unstable, today one thing, tomorrow another. All this is clearly and effectively brought out by our author.

In his treatment of the subject matter of Chapter XI, "The Process of Digestion in the Green Plant," the author points out the fact that the food requirements of the protoplasm of plants and animals are alike. His account of enzymes and the rôle of enzymes in the digestion of plant-food materials is a comprehensive summary of the subject as it has been developed through the research of scores of plant physiologists and biochemists throughout the world. Here, again, is subject matter of great importance to the phytopathologist and the agronomist. Often, when seed of corn, wheat, flax, or other plant is sown or planted, there arise many abnormal plants and not infrequently there is considerable seedling mortality from one cause or another. Possibly the seed is inherently weak, poor in the enzymes necessary to digestion. More often, however, the lack of seedling vitality is traceable to conditions inimical to catalytic action or favorable to fungous invasion and the consequent consumption of the food materials stored up in the seed or other plant structure.

Chapters XII and XIII, entitled "The Translocation of Materials in Plants" and "The Process of Respiration in Plants," respectively, should prove helpful to those engaged in agronomic and plant-pathologic research. It is obvious that a more exhaustive knowledge of the phenomena discussed in these two chapters might dissipate much of the mystery and mental fog

that make difficult and slow our progress in studies on winter hardiness of alfalfa, wheat, and other crop plants. Such knowledge might facilitate our progress in the investigation of such obscure physiologic derangements as straighthead of rice, internal tissue degeneracies, premature decline of vitality and loss of germinability in seeds of various plants, spindle tuber of the potato, floret sterility in many of our crop plants, phloem necrosis, storage rots, etc. Likewise, the 14th and final chapter of this important work contains much that cannot be other than helpful to the student of growth phenomena and the factors that promote or inhibit growth.

The reader of this work on plant physiology will undoubtedly be impressed by the fact that it is first of all what will surely prove to be a useful compilation of information on the various functions of the growing plant and the factors affecting one way or another the plant as a living entity. If he employes it in the classroom he will find it far more useful as a reference book than as a laboratory or classroom text. Its usefulness as a reference book could have been increased had the 36 pages, devoted to more or less orthodox test or examination questions, been filled with pertinent and needed illustrations of apparatus, graphs, and other matter supplementary to the text. Another and revised edition should take cognizance of such desirable improvements and should be given the benefit of more detailed attention to matters of grammatical construction and clarity and comprehensiveness of table legends.—H. B. HUMPHREY, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.